

cDNA cloning, recombinant expression and characterization of polypeptides with exceptional DNA affinity

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Received December 12, 1997; Accepted January 14, 1998

DDBJ/EMBL/GenBank accession nos X95591, X95592

ABSTRACT

Polypeptides remaining tightly associated with isolated genomic DNA are of interest with respect to their potential involvement in the topological organization and/or function of genomic DNA. Such residual DNA–polypeptide complexes were used for raising monoclonal antibodies by *in vitro* immunization. Screening of a murine λ gt11 cDNA library with these antibodies released a positive cDNA (MC1D) encoding a 16 kDa polypeptide. The cloned homologous human cDNA (HC1D) was identified in the dbest data base by partial sequence comparison, and it was sequenced full length. The cDNA-derived amino acid sequences comprise nuclear location signals but none of the known DNA-binding motifs. However, the recombinantly expressed proteins show *in vitro* DNA binding affinities. A polyclonal antiserum to the recombinant MC1D protein immunostains sub-nuclear structures, and it detects a residual 16 kDa polypeptide on western blots of DNA digests. These results support the conclusion that the cloned cDNAs reflect mRNAs encoding one of the chemically-resistant polypeptides which can be detected in isolated genomic DNA by sensitive techniques, e.g. by ¹²⁵Iodine labeling and SDS–PAGE.

INTRODUCTION

Epigenetic regulation of gene expression including phenotypic expression involves local or extensive alterations of chromatin structure (1,2). This view is experimentally supported by recent results of chromosome painting with chromosome-specific composite and gene-specific probes (3,4). The latter results show that interphase chromosomes and individual genes occupy distinct territories and sites in the interphase cell nucleus. Further support for this hypothesis is expected to come from the

biochemical characterization of DNA–polypeptide complexes potentially involved in the 3-dimensional genome organization, e.g. in the configuration of the volumes and shapes of the territories occupied by interphase chromosomes. In this context those polypeptides are of special interest, which are able to form either permanent or transient covalent complexes with DNA. Several studies characterized *in situ* existing chemical bonds between polypeptides and DNA, e.g. combined and prolonged protease/nuclease treatment of purified genomic DNA releases complexes containing phosphoester bonds between tyrosine residues of oligopeptides and nucleotides (5–8). However, the origin of these oligopeptides is still unknown. They could reflect remnants of the well known transient covalent complexes between topoisomerases and DNA. Other candidates are the polypeptides which are of lower molecular weight (16–68 kDa) than topoisomerases (91 kDa, swiss: TOPO1_Mouse; 173 kDa swiss: TOPO2_Mouse) and which are displayed by sensitive analytical techniques, e.g. by ¹²⁵Iodine treatment of highly purified DNA (9). In this paper we describe the molecular cloning and recombinant expression of cDNAs suggestively encoding one of these resistant polypeptides. Moreover, we present data on the DNA binding activity of the recombinant protein.

MATERIALS AND METHODS

Isolation of genomic DNA

DNA was isolated from Ehrlich ascites cells by prolonged alkaline (1 M NaOH) cell lysis (5) or by cell lysis in SDS buffer containing 50 μ g/ml of proteinase K (Merck) (10). Cell lysates were extracted with phenol (three times) and DNA was precipitated as described previously (5,10).

¹²⁵Iodine-labeling of residual proteins in genomic DNA

Samples (1.25 mg) of alkali-denatured DNA dissolved in 50 μ l TE (10 mM Tris–HCl, 5 mM EDTA, pH 7.4) were mixed with

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125 μ Ci of Na¹²⁵I (17.4 Ci/mg, NEN) in 100 μ l H₂O and 10 μ l chloramin T solution (12 mg/ml H₂O). After 5 min at room temperature β -mercaptoethanol (10 μ l) and a solution of unlabeled NaJ (10 μ l, 20 mg/ml TE 0.1) were added. Aliquots were heat-degraded (at least 5 min, 95°C) in sample buffer (11) and submitted to SDS–polyacrylamide (12% w/v) gel electrophoresis. The dried gels were exposed to Kodak X-Omat X-ray films. Another portion of the radio-iodination mixture was extracted with phenol (three times) and the DNA (associated with radiolabel) contained in the aqueous phase was ethanol-precipitated. The dried pellet was resuspended in SDS-containing buffer and submitted to Sephadex™ (Pharmacia) chromatography as described in the legend of Figure 1. Pooled fractions were ethanol-precipitated and submitted to SDS–PAGE described above.

Monoclonal antibodies prepared by *in vitro* immunization

DNA (50 mg) isolated from Ehrlich ascites cells by phenol extraction of SDS/proteinase K lysates were digested with micrococcal nuclease (Boehringer-Mannheim) under dialysis conditions (Schleicher and Schüll, Ultra Thimbles). The residual material was precipitated with acetone and the precipitated material was collected by centrifugation (10 min, 10 000 g). The pellet was resuspended in water (4°C) and re-centrifuged as described before. The material insoluble in water was suspended in 400 μ l of PBS, and dispersed by sonication (4°C). *In vitro* immunization was carried out essentially as described by Reading (12). Briefly, a single cell suspension (1 \times 10⁸ cells) in 10 ml serum-free DMEM (s-DMEM) prepared from the spleen of an 8 week old Balb/c mouse was incubated with the antigen for 1 h at 37°C and 10% CO₂ followed by addition of 10 ml of thymocyte-conditioned medium, prepared according to (12), and 10 ml s-DMEM. After incubation for 5 days at 37°C and 10% CO₂, cells were washed with s-DMEM and fused with P3-X63-Ag 8.643 mouse myeloma cells as described in detail (13). Supernatants of hybridoma cell clones were assayed for specific monoclonal antibodies by ELISA using 'nuclear matrix' proteins as antigen. This protein fraction was prepared as described previously in detail (14). Briefly, nuclei of Ehrlich ascites cells were extracted after DNase I digestion with buffers containing 2 M NaCl. The residual insoluble proteins were dissolved in SDS-containing buffer and adsorbed to the wells of microtiter plates. ELISA positive supernatants were additionally tested for specificity by western blots using again 'nuclear matrix' proteins as antigen. Analysis by a monoclonal antibody isotyping kit (Boehringer-Mannheim) revealed that the antibodies of interest were of the IgM class.

Plaque screening, recloning, sequencing and data base searches

λ gt11 recombinants of murine RNA-based cDNA were screened as plaques on a lawn of *Escherichia coli* Y1090 by means of hybridoma culture supernatants supplemented with 1% w/v BSA. The procedure was essentially that recommended by Huynh *et al.* (15). As a second antibody we used ¹²⁵Iodine-labeled sheep anti mouse Ig (Amersham). Positives were plaque-purified and the inserts were recloned in the pBluescript vector (Stratagene). DNA was sequenced according to Sanger *et al.* (16) by means of a DNA sequencing kit from Pharmacia. Recently, data base searches (17) released a partial homologous sequence to the murine C1D sequence. The human cDNA clone (pHC1D) was kindly supplied

to us by the IMAGE consortium (clone ID 111003 5'). Its full length nucleotide sequence revealed the homology with the murine MC1D sequence.

Recombinant expression of the MC1D protein and preparation of a polyclonal antiserum

The nucleotide sequences encoding the 16 kDa proteins were PCR amplified and restriction sites were added during amplification: *KpnI* (5'-end) and *SalI* (3'-end). The amplified fragment was cloned into the *KpnI*–*SalI* sites of the pQE 30 vector, expressed in *E.coli* M15 cells and the MC1D protein was isolated and purified after lysis of the bacterial cells by adsorption to Ni-NTA resin according to the instructions of the producer (Qiagen™). The protein was released from the resin by EDTA in 8 M urea and immediately dialysed against TE 0.1 (Schleicher and Schüll, Ultra Thimbles). During dialysis the antigen becomes completely insoluble and can be collected by centrifugation. Four 100 μ g pellets of the recombinant MC1D protein were used for successive subcutaneous injections of rabbits at intervals of 3 weeks. The antigen was emulsified in complete Freund's adjuvant (first injection) or in incomplete Freund's adjuvant in successive injections. Immune sera were prepared 2 weeks after the last injection (Eurogentec, Seraing, Belgium).

Immunocytochemistry

Cells settled on lysine coated slides were fixed in cold (4°C) ethanol, washed with PBS and incubated in PBS/1% BSA with the antiserum to the MC1D protein (dilution 1:500, 60 min at room temperature). After several washes with PBS, the cells were incubated under the same conditions with FITC-conjugated affinity-pure anti-rabbit IgG, Jackson (dilution 1:200). After three washes with PBS, the specimens were covered with a cover slip, inspected in the fluorescence microscope and photographed.

Immunochemical detection of resistant polypeptides in DNA digests

Batches (~4 mg) of DNA isolated from Ehrlich ascites cells by prolonged alkaline (1 M NaOH) cell lysis (5) were digested with Benzonase™ under dialysis conditions (50 mM Tris–HCl, pH 8.0, 0.1 mM MgCl₂), concentrated by lyophilisation and submitted to SDS–polyacrylamide (12% w/v) gel electrophoresis. Gel lanes were either silver-stained or blotted to nitrocellulose membranes which were probed with the polyclonal serum against the recombinant MC1D protein (dilution 1:100 in PBS). The second antibody was ¹²⁵Iodine-labeled anti-rabbit Ig (Amersham).

DNA binding, mobility shifts and electron microscopy

DNA (genomic DNA, supercoiled plasmid, linearized plasmid) was mixed with different amounts of recombinant MC1D protein dissolved for various times in buffered (0.1 M NaH₂PO₄, 0.01 M Tris–HCl, pH 8.0) 8 M urea. Dialysis of such mixtures or dilutions resulting in urea concentrations below 0.5 M induce the MC1D protein binding to DNA. Dialysed mixtures (specified in figure legends) were used for DNA mobility shift experiments, turbidity measurements (not shown) and electron microscopy. For electron microscopy, DNA was directly mounted to mica (10 mM Tris–HCl, pH 7.3, 1 mM EDTA, 50 mM MgCl₂) and stained with 1% (w/v) UO₂-acetate. Shadowcasting was performed under 5° by Pt/C = 95/5.

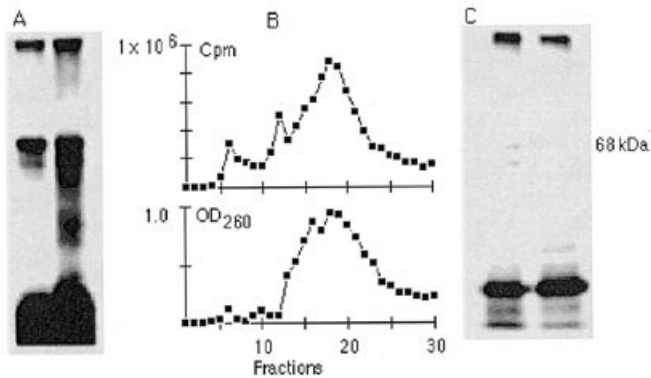


Figure 1. Polypeptides associated with alkali-denatured and phenol-extracted eukaryotic DNA. (A) Autoradiography of an SDS-polyacrylamide gel (12%, w/v) loaded with different amounts of ¹²⁵Iodine-labeled polypeptides co-isolating with genomic DNA. The left lane shows in addition to undefined radiolabeled material retained in the gel pocket mainly polypeptides which are ~68 kDa in size. On an overloaded lane (right lane) additional radiolabeled polypeptides are detected (~54 kDa, ~30 kDa). Polypeptides migrating close to the buffer front are not detectable on such gels because they are overshadowed by undefined radiolabeled materials accumulating at the buffer front. (B) Co-chromatography (Sephadex G50) of ¹²⁵Iodine-labeled polypeptides with oligodeoxynucleotides (OD₂₆₀). Following ¹²⁵Iodine-labeling of the polypeptides associated with isolated DNA the reaction mixture was phenol extracted three times, ethanol precipitated and submitted to a Sephadex G50 column. Elution buffer was 50 mM Tris-HCl, pH 7.5, 10 mM EDTA and 150 mM NaCl, 0.1% SDS. Aliquots of the fractions were assayed for radioactivity and OD₂₆₀. (C) SDS-PAGE of ¹²⁵Iodine-labeled polypeptides after Sephadex G50 chromatography. Fractions 10–20 (left lane) and 20–30 (right lane) were pooled, ethanol precipitated and submitted to SDS-PAGE (12%, w/v). The figure shows an autoradiography of the gel.

RESULTS

Residual polypeptides tightly bound to isolated genomic DNA

Figure 1A shows ¹²⁵Iodine-adsorbing material at DNA prepared by alkaline cell lysis and repeated phenol extractions. Since DNA itself does not adsorb Iodine under the labeling conditions applied, it appears that highly purified DNA is still associated with polypeptides containing tyrosine residues which are the acceptors of Iodine. The significance of these radio-iodinated polypeptides is critical. Due to the sensitivity of the method radio-iodinated polypeptides detected in DNA preparations could reflect impurities contained in buffers and other substances used for the DNA isolation and for the radio-iodination procedure. Consequently, the specific interaction of radio-iodinated polypeptides with DNA has to be verified by additional purification steps, e.g. by extraction of the DNA-independent radiolabel with phenol. Additional Sephadex chromatography shows that an amount of the radiolabel is not removed from DNA during phenol extractions. Most of the remaining radiolabel appears in the void volume of Sephadex G25 columns (not shown). Sephadex G50 chromatography shows that the profile of the radiolabel parallels the OD₂₆₀ profile which points to a specific interaction of radio-iodinated polypeptides with oligonucleotides (Fig. 1B). Ethanol precipitation of the material contained in fractions with high OD₂₆₀ adsorption and its analysis by SDS-PAGE shows that the bands at 54–68 kDa are almost completely lost during this purification process (Fig. 1C). On the other hand, a radiolabeled component migrating like a

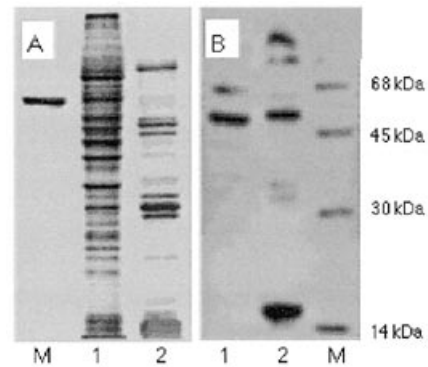


Figure 2. Western blot probed with the monoclonal antibodies used for λ gt11 plaque screening. (A) Coomassie blue-stained SDS-polyacrylamide gel (12%, w/v) showing a marker protein (M, 68 kDa), total cytoplasmic proteins (1) and nuclear proteins (2) from Ehrlich ascites tumor cells. (B) Autoradiography of the blot of a parallel gel section loaded with total cytoplasmic proteins (1), nuclear proteins (2) and ¹⁴C-labeled marker proteins (M) (Amersham). The first antibody was a mixture of the culture supernatants of two hybridoma cell lines (DP 1 and DP 2). The second antibody was ¹²⁵Iodine-labeled anti-mouse Ig. The immuno complex detected in the cytoplasmic fraction reflects a reaction of the ¹²⁵Iodine-labeled anti-mouse Ig with mouse immunoglobulins attached to the Ehrlich ascites cells harvested from the peritoneal cavity.

15–20 kDa polypeptide appears to be enriched if equal amounts of radiolabel are submitted to the gels shown in Figure 1A and C. The loss of the components migrating like 54–68 kDa polypeptides does not rule out their significance because large polypeptides stably associated with small pieces of DNA may be lost in the phenol phase. However, the enrichment of the 15–20 kDa component confirms the existence of polypeptides which are alkali and phenol-stably bound to DNA and which are unrelated to the much larger topoisomerases. It is obvious that the amount of this polypeptide surviving the entire procedure is too low for a successful biochemical isolation. Accordingly, a cDNA cloning approach appeared to be the only promising strategy for the characterization of this polypeptide at the sequence level.

Antibodies to residual polypeptides

The quantity of the polypeptides which can be released from phenol-extracted genomic DNA by nuclease digestion was sufficient for the *in vitro* immunization procedure, however, this amount of polypeptides was too short for the screening and clonal selection of hybridomas. Thus, for the screening procedure a more abundant protein fraction was applied which was expected to comprise the antigens of interest as well. This expectation was reasonable because the polypeptides co-isolating with DNA are insoluble after nuclease digestion. Accordingly, they become a portion of the fraction of insoluble nuclear proteins which is termed 'nuclear matrix'. Consequently, hybridoma clones were screened for antibodies against 'nuclear matrix' proteins isolated by DNase I digestion of Ehrlich ascites cell nuclei followed by extraction with high salt buffer (14). In order to avoid the selection of clones producing DNase I specific antibodies, the polypeptides used for immunization were released from DNA by micrococcal nuclease. Eight hybridoma cell lines (DP 1–8) were found which produced antibodies to nuclear proteins which was

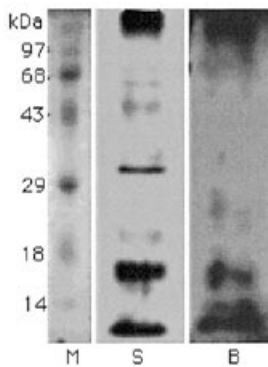


Figure 6. Relation between the cDNA-encoded MC1D protein and a 16 kDa protein co-isolating with DNA. M, marker lane loaded with pre-stained protein markers. S, silver-stained SDS-PAGE (12%, w/v) loaded with BenzonasTM-digested DNA (4 mg). B, identical with lane S, however, this lane was blotted to nitrocellulose which was probed with the antiserum to the recombinant MC1D protein and ¹²⁵Iodine-labeled anti rabbit Ig (Amersham). The site of the BenzonasTM band on lane S between 29 and 43 kDa is not immunostained on lane B which points to the specificity of the immunoreaction.

and human cells. The fibrogranular distribution of the antigen in nuclei of human fibroblasts is shown in Figure 5.

On western blots, the polyclonal antiserum immunoreacts specifically with two cellular antigens (Fig. 3B). One of these antigens is of the expected size (16 kDa), however, the 32 kDa antigen cannot be correlated with the length of the unique mRNA hybridizing with the radiolabeled cDNA (Fig. 3A). The relative molecular masses of the two cellular antigens and a missing mRNA for the 32 kDa antigen point to the side by side existence of a monomeric form of the protein with an SDS-resistant homo dimer molecule. Since the recombinant MC1D protein migrates on SDS-polyacrylamide gels in its monomeric form (Fig. 3C) it must be concluded that the dimerization of the 16 kDa cellular protein is caused by a post-translational modification.

It is obvious that only the monomeric form of the protein is detected in chemically resistant DNA-protein complexes (Figs 1C and 6). This suggests the specific binding of the monomeric form to DNA. Alternatively, dimeric molecules may be cleaved during the DNA isolation procedure which involves prolonged treatments with alkali and phenol.

Relation between the cDNA-encoded 16 kDa MC1D protein and the cellular 16 kDa protein co-isolating with DNA

The identity of the cDNA-encoded proteins with cellular proteins forming chemically resistant DNA-protein complexes cannot be verified at the sequence level. It is obvious that the quantities of the complexes co-isolating with total DNA are too small for the identification of the complex-involved polypeptides by microsequencing (Figs 1 and 6). Alternatively, it was investigated whether a sensitive immunanalytical approach could reveal the relationship between the cDNA-encoded proteins and the polypeptides involved in the stable complexes. It was found that digests of several milligrams of DNA are required to release quantities of the cellular 16 kDa protein which can be visualized on silver-stained SDS-polyacrylamide gels (Fig. 6, S) and immunostained by means of the polyclonal MC1D antiserum and ¹²⁵Iodine-labeled anti-

rabbit Ig (Fig. 6, B). Although the immunochemical reaction is not strong it is specific because the much more abundant BenzonasTM used to digest the DNA, present on the same blot, is not immunostained by this procedure.

The cellular antigen immunoreacting with the MC1D antiserum is easily detected in nuclei (Fig. 5) and on western blots of total cellular proteins (Fig. 3B). Since the immunoreaction with the DNA digests is not strong (Fig. 6, B) it follows that either most of the complexes are lost during the DNA isolation procedure or that only a small fraction of the cellular protein is directly involved in the chemically resistant DNA-polypeptide complexes.

DNA binding capacity of the recombinant proteins

The potential cognate binding site and the physiological conditions for the chemically stable interaction of the cellular protein with DNA are unknown. However, most sequence-specific DNA binding proteins show sequence-unrelated, but significant DNA affinities. Accordingly, experiments were designed which could reveal such basic DNA affinities of the recombinant C1D proteins.

The recombinantly expressed C1D proteins are readily soluble in 8 M urea (10 mg/ml) but completely insoluble in buffers containing <0.5 M urea. Consequently, the proteins precipitate during dialysis or during dilution with buffers. However, in the presence of genomic DNA, supercoiled or linear plasmid DNA the proteins remain in solution during dialysis or dilutions. This 'solubility' of the recombinant proteins in buffers is due to their binding to DNA molecules. Nuclease digestion of the complexes releases the protein in its insoluble form which can be followed by the increase of turbidity at OD₆₀₀ (not shown).

Binding of the recombinant proteins to supercoiled or linearized plasmid DNA induces DNA mobility shifts on agarose gels (Fig. 7A and B). However, the capacity to induce the electrophoretically stable complexes is restricted to the freshly isolated proteins or to the proteins stored by suspension in non-denaturing buffers at -20°C. Their potential to form the electrophoretically stable DNA-protein complexes is lost with a half life period of ~10 days when stored at 4°C in 8 M urea. Recombinant C1D proteins kept for 2 months in 8 M urea at 4°C are still kept in 'solution' by DNA in non-denaturing buffers, however, they have completely lost the capacity to induce DNA mobility shifts (Fig. 7C) which indicates that the electrophoretically stable complexes shown in Figure 7A and B are not based on an unspecific DNA-protein interaction. The loss of the capacity to induce DNA mobility shifts is, suggestively, the result of a known chemical modification of proteins occurring in concentrated urea solutions, e.g. carbamylation of amino groups by cyanate. This result shows that free amino groups are involved and apparently essential for the formation of electrophoretically stable DNA-protein complexes.

Electronmicroscopical inspection of the complexes formed between the recombinant proteins and plasmid DNA point to a cooperative binding mechanism. The micrographs exhibit a side by side existence of apparently protein-free plasmid molecules and those which are associated with large protein clusters (Fig. 8). This suggests that recombinant C1D protein molecules bind preferentially to existing C1D-DNA complexes.

The *in vitro* formed complexes between the recombinant C1D proteins and DNA are not phenol-stable. So far, attempts to induce this chemically stable DNA-C1D protein interaction *in*

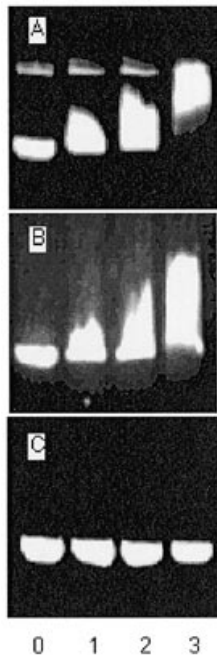


Figure 7. DNA mobility shifts induced by complex formation between the recombinant MC1D protein and plasmid DNA. (A) Supercoiled plasmid DNA (3 kb) was mixed with freshly isolated recombinant MC1D protein dissolved in buffered (0.1 M NaH_2PO_4 , 0.01 M Tris-HCl, pH 8.0) 8 M urea. Protein-DNA ratios were: lane 0, no protein added; lane 1, one protein molecule per 30 bp of DNA; lane 2, one protein molecule per 20 bp of DNA; lane 3, one protein molecule per 10 bp of DNA. The mixtures were dialysed against 1000 vol TE 0.1 (4°C). Aliquots containing 1 μg of DNA were submitted to a 0.5% (w/v) agarose gel. (B) Linearized plasmid DNA (3 kb) was mixed with freshly isolated recombinant MC1D protein, dialysed and submitted to agarose gel electrophoresis as described under (A). (C) Linearized plasmid DNA (3 kb) was mixed with recombinant MC1D protein which had been stored in buffered 8 M urea for 2 months at 4°C. The protein-DNA ratios, dialysis conditions and agarose gel electrophoresis were as described (A) and (B).

vitro, e.g. by incubation of the complexes with energy-rich compounds, have not yet been successful.

DISCUSSION

The identification of new DNA-binding proteins on the genetic level is of interest because the proteins potentially involved in changes of chromosome structure and in 'marking' sections of DNA chemically without changing the genetic code (1,2) are not yet identified. By combined immunoanalytical and recombinant DNA technology murine and human cDNAs were cloned encoding DNA-binding proteins missing any of the known DNA binding motifs specified in data bases (18). Accordingly, these proteins represent a new type of DNA binding proteins which is of interest in itself.

This work was initiated by the results of previous investigations on chemically resistant DNA-protein complexes. It has been shown that a persistent fraction of polypeptides is inevitably co-purified with eukaryotic DNA which points to covalent complexes between nuclear proteins and DNA (9). While phosphate-linked DNA-peptide bonds have been shown to exist in DNA preparations (5-8) little is known about the size, nature and function of the polypeptides involved in such chemically

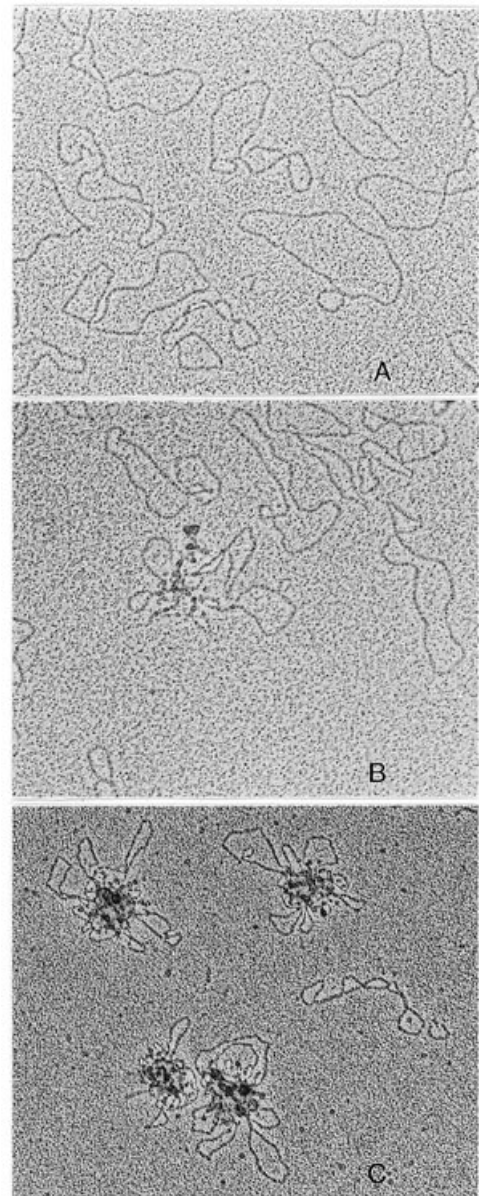


Figure 8. Electron microscopical visualisation of the complexes formed between a plasmid (3 kb) and the MC1D protein. (A) Plasmid DNA in mostly supercoiled conformation; control without MC1D protein. (B) Supercoiled plasmid DNA with 1 molecule of C1D protein per 30 bp of DNA (the same sample as shown in Fig. 7A, lane 1). (C) Supercoiled plasmid DNA with 1 molecule of C1D protein per 10 bp of DNA (the same sample as shown in Fig. 7A, lane 3).

stable DNA-protein complexes. This is mainly due to the small quantities of complexes which are only revealed by sensitive analytical techniques. It is obvious that the polypeptides involved in these complexes cannot be identified by biochemical methods, e.g. by micro-sequencing. Although an unknown amount of these complexes may be lost during the DNA isolation procedure, e.g. in the phenol phase, it is evident that these complexes are not major cellular components. However, even minute amounts of potentially site-specific DNA-protein complexes could be involved in important biological functions, e.g. in the epigenetic regulation

of gene expression. Accordingly it seemed to be worthwhile to try the molecular cloning of cDNAs encoding proteins defined by their unusually tight interaction with DNA.

Although the identification at the sequence level is missing there is evidence that we cloned human and murine cDNAs encoding a 16 kDa protein involved in chemically resistant DNA-protein complexes. This suggestion is based on the general design of this work and the identical sizes of the resistant cellular proteins and the cDNA-encoded proteins. Moreover, antibodies to the murine recombinant protein could detect the resistant protein on a western blot of the residual polypeptides released from DNA by nuclease digestion. Finally, the cDNA-encoded proteins show the expected cellular location and function consisting in a DNA-binding activity. It is highly unlikely that a false-positive clone encodes an antigenic protein with the expected size, cellular location and function. Accordingly, the available criteria point to the identity of the cDNA-encoded proteins and one of the proteins co-isolating with phenol-extracted DNA.

Diverging characteristics of the cDNA-encoded proteins and the corresponding cellular 16 kDa proteins are suggestively caused by cellular events, conditions and protein modifications. For example, the cellular protein tends to dimerize which seems to be the result of a well known cellular transamidating activity (19). The formation of chemically-resistant bonds between the proteins and DNA, requires expectedly, cellular conditions which could not yet be achieved in *in vitro* experiments.

Further studies by means of the new molecular probes including vector-encoded overexpression, are expected to supply more information on the function of these complexes and especially on their potential involvement in cell type specific patterns of gene expression.

ACKNOWLEDGEMENT

T.G. is grateful for a fellowship of the German Cancer Research Center, Heidelberg.

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