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Nuclear Localization and DNA Interaction of Protein Disulfide Isomerase ERp57 in Mammalian Cells

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Abstract Protein disulfide isomerase ERp57 is localized predominantly in the endoplasmic reticulum, but is also present in the cytosol and, according to preliminary evidence, in the nucleus of avian cells. Conclusive evidence of its nuclear localization and of its interaction with DNA in vivo in mammalian cells is provided here on the basis of DNA–protein cross-linking experiments performed with two different cross-linking agents on viable HeLa and 3T3 cells. Nuclear ERp57 could also be detected by immunofluorescence in HeLa cells, where it showed an intracellular distribution clearly different from that of an homologous protein, located exclusively in the endoplasmic reticulum. Mammalian ERp57 resembles the avian protein in its recognition of S/MAR-like DNA sequences and in its association with the nuclear matrix. It can be hypothesized that ERp57, which is known to associate with other proteins, in particular STAT3 and calreticulin, may contribute to their nuclear import, DNA binding, or other functions that they fulfil inside the nucleus. J. Cell. Biochem. 85: 325–333, 2002.

Key words: protein disulfide isomerase; ERp57; PDI; nuclear proteins; DNA binding

ERp57, also known as ERp60, ERp61, or GRP58 [Mazzarella et al., 1994; Bourdi et al., 1995], is a member of the protein disulfide isomerase family of enzymes [Freedman et al., 1994], and was found originally in the endoplasmic reticulum, together with PDI, the best-known member of the family. A variety of functions have been attributed to ERp57, i.e., phospholipase C [Bennett et al., 1988], protease [Urade and Kito, 1992], carnitine palmitoyl transferase [Murthy and Pande, 1994], and reductase [Srivastava et al., 1991] activities. The phospholipase C activity has been definitively ruled out [Mazzarella et al., 1994], but the reductase activity has been repeatedly observed [Srivastava et al., 1991; Hirano et al., 1995]. In any case, the presence of two thioredoxin-like active sites leaves little doubt that its function is related to the redox properties of these sites. More recently, ERp57 in its endoplasmic reticulum location has been shown to have a role in the correct folding and in the disulfide bonds rearrangement of misfolded glycoproteins [Elliott et al., 1997]. It has also been demonstrated that for this function, the formation of a complex between ERp57 and either calnexin or calreticulin is required [Zapun et al., 1998; Oliver et al., 1999]. These two proteins associate with ERp57 also in the assembly of the major histocompatibility complex I [Lindquist et al., 1998].

Evidence has been presented indicating other functions and/or other localizations of this protein. Thus, the activation of STAT3 in the cytosol has been shown to be accompanied by the formation of a multiprotein complex containing STAT3, ERp57, and other proteins [Ndubuisi et al., 1999]. Activated STAT3 is known to enter the nucleus and act as a transcription factor, binding to specific DNA sequences. STAT3 is known to form complexes also in the nuclear compartment with other proteins, but no evidence exists yet for the presence of ERp57 in these nuclear complexes. Considering the variety of consequences originating from STAT3 activation and from its nuclear import, it would certainly be important to identify all of its associated nuclear proteins. A necessary

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premise to the identification of ERp57 as one of these associated proteins should be the unequivocal demonstration of its presence in the nucleus.

At least another ERp57-interacting protein has recently been shown to have a nuclear localization, i.e., calreticulin. The presence of calreticulin in the nucleus has long been denied, but experiments performed with the GFP-fusion protein [Roderick et al., 1997] and, more recently, the demonstration of the involvement of calreticulin in the nuclear export of some proteins [Holaska et al., 2001] have left no doubt of its nuclear localization.

Thus, if ERp57 is present also in the nucleus, its capacity to associate with at least two identified nuclear components might exert a regulatory effect on a variety of biological processes. We have previously described ERp57 as a component of the internal nuclear matrix in chicken cells [Altieri et al., 1993] and have shown that *cis*-DDP (*cis*-diammine dichloro platinum) induces the cross-linking of ERp57 to DNA in chicken liver nuclei. Moreover, the avian protein was shown to recognize DNA sequences resembling the scaffold/matrix associated regions (S/MARs) [Ferraro et al., 1999].

However, in order to assess the general importance of a possible nuclear localization of ERp57, and in particular to verify this localization in those organisms where STAT3 activity has been described, it is necessary to take into consideration the mammalian cells. Actually a nuclear localization of this protein has been reported in rat spermatids and spermatozoa [Ohtani et al., 1993] and in human blood cells [Gerner et al., 1999].

It must be stressed, however, that most of the afore-mentioned studies regarding either the avian or the mammalian protein have been performed by analyzing the content of isolated nuclei, which, even when purified, might be contaminated during their preparation by the abundant ERp57 present in the endoplasmic reticulum. This represents a common difficulty for all proteins present in small amounts in nuclei, but with high abundance in the endoplasmic reticulum. Immunofluorescence methods, in particular, suffer from this problem, because the strong signal from endoplasmic reticulum usually hides the nuclear signal.

A DNA–protein cross-linking method could therefore be the approach of choice. While we have already exploited this method for the identification of nuclear ERp57 [Ferraro et al., 1999], it should be noted that the cross-linking was performed on isolated nuclei, which could have been contaminated as mentioned before, and only on material of avian origin.

Therefore, in order to provide a conclusive proof for the presence of ERp57 in nuclei, and particularly in those of mammalian cells, we performed the DNA–protein cross-linking on intact, viable cells from murine and human origin, using two different cross-linking reagents, i.e., *cis*-DPP and UV-irradiation. With this approach we have been able to demonstrate that ERp57 is present also in the nuclei of cultured mammalian cells and that it interacts directly with DNA. Furthermore, in HeLa cells the amount of the protein in nuclei was such that its presence could be detected also by immunofluorescence.

MATERIALS AND METHODS

Materials

HeLa and 3T3 cells were grown to 70–80% confluence at 37°C in 5% CO_2 in DMEM medium supplemented with 10% fetal bovine serum, 100 U/ml of penicillin, and 100 μg/ml of streptomycin.

A polyclonal anti-ERp57 antibody was prepared in rabbits by the use of the protein purified from pig liver as antigen. In the course of the experiments described below, it was demonstrated that this antibody was entirely specific for ERp57. The anti-PDI polyclonal antibody was a commercial product (Stressgen).

ERp57 was purified from a pig liver microsomal preparation following the method of Altieri et al. [1993]. Human recombinant ERp57 protein was obtained [F. Altieri, unpublished results] as a GST-ERp57 fusion protein from a plasmid kindly provided by Dr. Bourdi.

DNA–Protein Cross-Linking by *Cis*-DDP

HeLa and 3T3 cells were treated for 2.5 h at 37°C with 0.5 mM *cis*-DDP in 1 ml/10^6 cells of *cis*-DDP buffer (0.25 M sucrose, 20 mM sodium phosphate, 5 mM KCl, 1 mM $MgSO_4$, 135 mM sodium acetate at pH 7.5). The cells were then washed with *cis*-DDP buffer containing 5 mM thiourea and scraped. The nuclei were purified by hypotonic lysis in 20 mM HEPES/NaOH pH 8, 10 mM KCl, 1.5 mM $MgCl_2$, 30 mM sucrose, 0.5 mM DTT (dithiothreitol), supplemented with an inhibitor cocktail (1 mM phenylmethyl

sulfonylfluoride; 10 μM amido-phenylmethyl sulfonyl fluoride; 0.2 mM L-1-chloro-3-(4-tosyl-amido)-4-phenyl-2-butanone; 0.15 μM aprotinin; 1 μg/ml E 64; 1 μM pepstatin; all from Roche), and 0.05% Triton X-100 [Lee and Green, 1990]. The nuclei were lysed with 5 M urea, 2 M guanidine-HCl, 2 M NaCl, 10 mM sodium phosphate at pH 7.5, and mixed with hydroxyapatite (1 g/10 mg DNA) [Ferraro et al., 1996]. The proteins non-cross-linked to DNA were eluted by washing the hydroxyapatite extensively with buffer A (5 M urea, 2 M guanidine–HCl, 2 M NaCl, 300 mM sodium phosphate at pH 7.5), then once with buffer B (5 M guanidine–HCl, 10 mM DTT, 300 mM sodium phosphate at pH 7.5) for 20 min at room temperature, and finally twice again with buffer A with incubations at 37°C for 30 min. The DNA-cross-linked proteins were then eluted by two incubations at 37°C for 90 min with 1 M guanidine–HCl, 1 M NaCl, 1.5 M thiourea, 150 mM sodium phosphate at pH 7.5. The inhibitor cocktail was present in each buffer.

DNA–Protein Cross-Linking by UV

The cells, grown on Petri dishes, were washed in PBS (phosphate-buffered saline, pH 7.4) and exposed to UV irradiation (254 nm) from a Spectroline hand-held lamp for 12 min at 4°C. The cells were then scraped, harvested by centrifugation, and washed twice with PBS. The nuclei, prepared as described above, were lysed in 50 mM Tris-HCl, 5 mM EDTA at pH 7.5 and were sonicated for 2 min (with Soniprep 150, MSE; Amplitudo 14 μm); this treatment produced DNA fragments of an average size of 1 kb. Sonicated nuclei were added with 0.3 M NaCl, 250 μg/ml DNAse-free RNAse A and the inhibitor cocktail, and incubated at 37°C for 90 min. This suspension, plus 2% SDS and 10 mM DTT, was centrifuged for 10 min at 10,000*g*. The clarified supernatant was used for gel-filtration chromatography. In a typical experiment, 1 ml of lysed nuclei (40 OD_{260}) was loaded on a Sephacryl HR 400 (Pharmacia) column (70 × 1.6 cm) [Ferraro et al., 1999] and eluted with 10 mM Tris-HCl, 0.5% SDS, 1 mM EDTA at pH 7.5 at a rate of 1 ml/min. The fractions containing DNA and having a constant 260/280 absorbance ratio were pooled and subjected to ethanol precipitation. The precipitate was collected, the DNA–protein complexes were dissolved in 50 mM Tris-HCl, pH 8, 4 M urea, 2 mM $MgCl_2$, and the DNA was digested by benzonase (Merck).

Immunofluorescence

HeLa or 3T3 cells grown on glass coverslip were fixed in 4% paraformaldehyde in PBS for 20 min, and permeabilized in 0.1% Triton X-100 for 15 min. The cells were then incubated for 1 h in 1% BSA (bovine serum albumin) in PBS, 1 h with rabbit sera (diluted 1:100 for anti-ERp57; 1:200 for anti-PDI) in 1% BSA in PBS, rinsed three times in PBS and then stained for 1 h with FITC-conjugated anti-rabbit IgG diluted 1:20 in 1% BSA in PBS. Cells were then rinsed twice with PBS, and stained with 0.1 μg/ml DAPI (4,6-diamidino-2-phenylindole) in PBS. After a brief wash in PBS, the cells were mounted in a specific medium for fluorescence (Vectashield) and observed, using a Zeiss 1.25, 40 × Neofluar objective. Control experiments were also carried out in which rabbit pre-immune serum was used instead of anti-ERp57.

Gel Shift

Mobility shift assay was performed with a 79 bp AT-rich oligonucleotide fragment (73% AT) corresponding to the sequence 1153–1231 of pSP65 plasmid (EMBL:CVPSP65, AC:X65329) and obtained by polymerase chain reaction (PCR) using ATCTTCACCTAGATCCT as forward primer and GTCAGACCAAGTTTACTC as reverse primer. The purified DNA fragment was labeled by means of a single cycle of PCR in the presence of [α-^{32}P] dATP. DNA binding was performed in 20 μl incubation mixture containing 20 mM Tris-HCl pH 7.5, 50 mM NaCl, 10% glycerol, 1 ng of radiolabeled DNA, and 1 μg of ERp57 in presence or absence of DTT or a 1,000-fold excess of non-labeled DNA. The DNA–protein binding mixtures were incubated for 1 h at 4°C and the resulting complexes were separated on 4.5% poly-acrylamide gels in 0.25 × TBE (25 mM Tris, 22.5 mM borate, 0.25 EDTA pH 8.0). After the gel had pre-run at 100 V for 1 h at 4°C, electrophoresis was performed at 180 V for 2.5 h at 4°C. Gels were air-dryed and exposed overnight to a Kodak BioMax XS film.

Other Procedures

The internal matrix from 3T3 and HeLa nuclei was prepared according to Kaufmann and Shaper [1984], with the modification of Stuurmann et al. [1990]. The proteins were analyzed by SDS–gel electrophoresis in 10% polyacrylamide, and by two-dimensional electrophoresis using Immobiline strips (Pharmacia) for the first

dimension. For the detection after Western blotting, the polyclonal anti-ERp57 antibody was used, followed by alkaline phosphatase-conjugated antirabbit IgG (Sigma) and a chemiluminescent substrate (Tropix).

RESULTS

Cross-Linking With *cis*-DDP

The proteins cross-linked to DNA by *cis*-DDP in HeLa and 3T3 cells were isolated by the use of hydroxyapatite, as described. The addition of thiourea in low amounts before the cell lysis was intended to block any reactive, monofunctional *cis*-DDP still present and therefore hinder the formation of artifactual cross-linking of the proteins during the subsequent steps. ERp57 was present among the cross-linked proteins from both cell types, as shown in Figure 1 (lane 6) and Figure 3 (lane 6). ERp57 from 3T3 cells was present in multiple forms. The minor components might originate from post-translational modifications or from partial degradation.

The protein was absent when control experiments were carried out by using the same method in the absence of *cis*-DDP, demonstrating the efficient separation of cross-linked and non-cross-linked proteins by the procedure used. The treatment of the cross-linked complexes with 10 mM DTT during the hydroxyapatite procedure indicates that ERp57 was cross-linked directly to DNA, and not through a disulfide bond formed with a different protein.

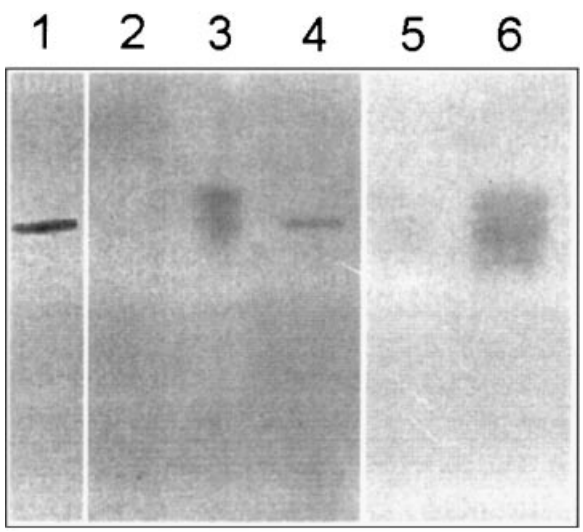

Fig. 1. SDS–gel electrophoresis and Western blots with anti-ERp57: proteins from 3T3 cells. **Lane 1**: Proteins from the internal nuclear matrix, from 9×10^5 cells. **Lane 2**: Control of the UV-cross-linking procedure (no irradiation). **Lane 3**: Cross-linked proteins from UV-irradiated cells (corresponding to 60 μg of DNA). **Lane 4**: Purified ERp57 (5 ng). **Lane 5**: Control of the *cis*-DDP cross-linking procedure (no *cis*-DDP treatment). **Lane 6**: Cross-linked proteins from *cis*-DDP treated cells (corresponding to 100 μg of DNA).

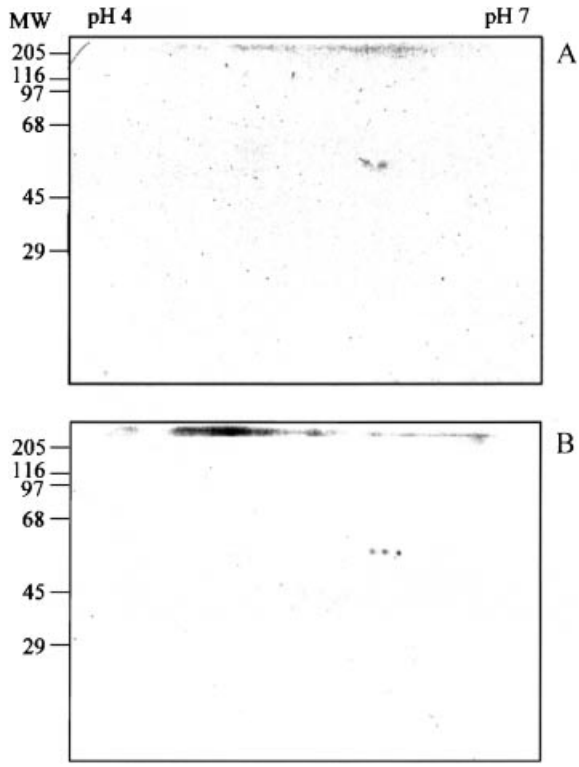

Fig. 2. Two-dimensional electrophoresis and Western blots with anti-ERp57. **A**: Purified ERp57. **B**: Cross-linked proteins from 3T3 cells treated with *cis*-DDP.

When the cross-linked proteins from 3T3 cells were analyzed by two-dimensional electrophoresis, the Western blot showed the presence of ERp57 in the expected position, i.e., in the 57 kDa/5.8 pI region (Fig. 2). No other protein reacted, demonstrating the specificity of the antibody used.

Using this method, proteins cross-linked to single-stranded nucleic acids are washed away from hydroxyapatite before the dissociation step with thiourea. Therefore ERp57 appears to interact with double-stranded DNA and, consequently, to be present in the nuclear compartment.

Cross-Linking With UV

HeLa and 3T3 cells have been also subjected to UV-irradiation. ERp57 was cross-linked to DNA in both cell types, as shown in Figure 1

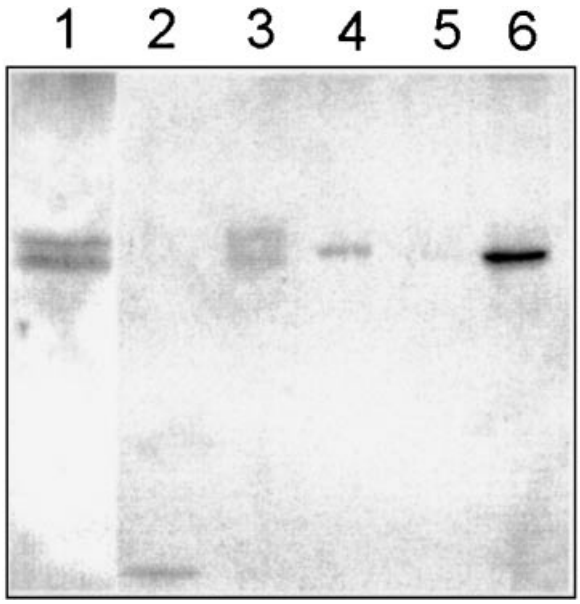

Fig. 3. SDS–gel electrophoresis and Western blots with anti-ERp57: proteins from HeLa cells. **Lane 1**: Proteins from the internal nuclear matrix, from 1.5×10^6 cells. **Lane 2**: Control of the UV-cross-linking procedure (no irradiation). **Lane 3**: Cross-linked proteins from UV-irradiated cells (corresponding to 100 μg of DNA). **Lane 4**: Purified ERp57 (5 ng). **Lane 5**: Control of the *cis*-DDP cross-linking procedure (no *cis*-DDP treatment). **Lane 6**: Cross-linked proteins from *cis*-DDP treated cells (corresponding to 100 μg of DNA).

(lane 3) and Figure 3 (lane 3). The multiple bands might originate from post-translational modifications or from an incomplete digestion of the DNA bound to the protein. Other cell lines (human HL-60 and murine F9-AC cl9) gave similar results (data not shown).

In these experiments the cross-linked- and non-cross-linked proteins were separated by the use of a gel filtration on a column of Sephacryl-400 HR, and again the efficiency of separation was tested by running control experiments, without any irradiation.

The UV-formed complexes were also treated with DTT, as was done with the *cis*-DDP-complexes. This again demonstrated that ERp57 binds DNA directly and not through a protein–protein interaction stabilized by a disulfide bridge. Since RNA was digested before the gel filtration, any protein cross-linked to RNA eluted together with the free proteins. It can be concluded that ERp57 is bound in vivo to DNA.

Considering the possible association between STAT3 and ERp57, and the finding of a small amount of STAT3 in the nuclear matrix of 3T3 cells [S. Coppari, unpublished data], the proteins cross-linked to DNA by UV in the same cell type were examined by Western blotting also for this protein. No STAT3 was found (data not shown), as expected, since only the activated form of the protein is known to bind DNA.

Subnuclear Localization of ERp57

ERp57 was identified as a nuclear matrix protein in avian nuclei [Altieri et al., 1993]. In order to verify that it has the same localization in mammalian nuclei, the internal nuclear matrix was prepared from purified nuclei of 3T3 and HeLa cells. The proteins from these preparations were fractionated by SDS–electrophoresis, and the Western blots revealed the presence of ERp57 (Fig. 1, lane 1, and Fig. 3, lane 1). While purified nuclei are expected to be contaminated with material from the endoplasmic reticulum, which is highly enriched in this protein, the internal nuclear matrix can be assumed to be free from such contaminations. Therefore, also in mammalian nuclei, ERp57 appears to be a component of the nuclear matrix.

The specificity of the antibody was confirmed by Western blotting of total nuclear proteins. When the antibody was pretreated with an excess of recombinant ERp57, the response of nuclear ERp57 was abolished (Fig. 4).

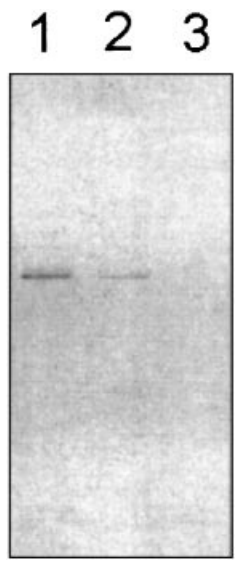

Fig. 4. Western blots of ERp57. **Lane 1**: Purified ERp57 from pig liver. **Lanes 2** and **3**: ERp57 in total nuclear extracts from HeLa cells. In lane 3, the anti-ERp57 antibody used had been pretreated overnight with an excess of recombinant GST-human ERp57.

Detection of Nuclear ERp57 by Immunofluorescence

Nuclear ERp57 could be revealed by immunofluorescence in growing HeLa cells (Fig. 5A). It should be noted that the anti-ERp57 antibody used did not react with any other nuclear protein, as described in the preceding paragraph and as shown in Figure 4.

PDI, which is considered a good marker for the endoplasmic reticulum, and which in fact appeared to be mostly or entirely extranuclear (Fig. 5C), showed a significantly different pattern.

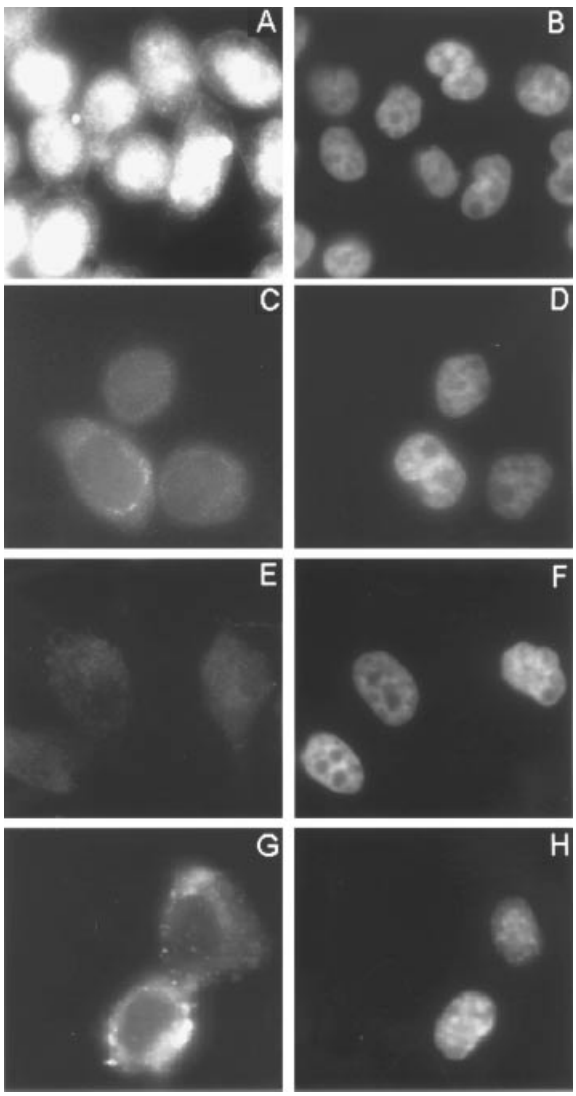

Fig. 5. Subcellular immunolocalization of ERp57 and PDI. ERp57 (**A**) and PDI (**C**) in HeLa cells. **E**: Preimmune serum on HeLa cells. **G**: ERp57 in 3T3 cells. **B**, **D**, **F**, and **H**: The same cells stained with DAPI.

Other mammalian cell lines failed to show nuclear ERp57 by immunofluorescence, while still revealing it by means of cross-linking (e.g., 3T3, Fig. 5G).

Gel Shift

Avian ERp57 was shown to bind with significant affinity to MAR-like DNA sequences [Ferraro et al., 1999]. To check if the mammalian protein has the same property, the DNA-binding capability of ERp57 purified from pig liver was tested by electrophoretic mobility shift assay with a radio-labeled AT-rich, MAR-like, DNA fragment. DNA-ERp57 interaction was clearly observed, while the radio-labeled DNA in the DNA–protein complex was completely displaced by the presence of an excess of the corresponding non-labeled DNA fragment (Fig. 6). Also the presence of 50 mM DTT in the incubation mixture strongly decreased the formation of DNA–protein complexes, showing that the mammalian protein, just as the avian one, interacts with DNA only in the oxidized state.

DISCUSSION

DNA–protein cross-linking is nowadays receiving a wide recognition as a safe method to detect DNA–protein interactions occurring in vivo. In the case of ERp57 this method can also afford a definite proof of the intranuclear localization of the protein, which until now has been based on evidence that could be subjected to criticism.

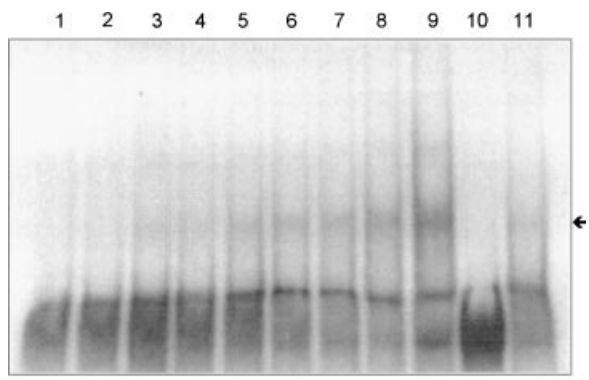

Fig. 6. Electrophoretic mobility shift assay of ERp57. Radiolabeled DNA fragments were incubated with ERp57. **Lane 1**: Control DNA fragment. **Lanes 2–9**: DNA + increasing amounts of ERp57 (10, 20, 50, 100, 200, 250, 500, and 1,000 ng, respectively). **Lane 10**: DNA + 1 μg of ERp57 + excess of unlabeled DNA. **Lane 11**: DNA + 1 μg of ERp57 + 50 mM DTT. The arrow indicates the DNA-ERp57 complex.

Two cross-linking reactions, taking place with entirely different mechanisms, have been performed on two types of viable, cultured cells of mammalian origin. *cis*-DDP has been known for a long time to be a very efficient cross-linking agent [Wedrychowski et al., 1986]. UV-irradiation is a physical zero-length cross-linking agent which can be considered the reagent of choice to identify the proteins directly interacting with DNA. The disadvantage is that some proteins, even when bound to DNA, escape the cross-linking or are cross-linked in low yields.

ERp57 was found to be complexed to DNA by both cross-linking agents. The possibility that this protein is not bound to DNA directly, but through the formation of a disulfide-stabilized heterodimer with another protein has been considered, but even in this event the demonstration of the nuclear localization of ERp57 would not be affected. In any case, the use of DTT under the conditions known to reduce the disulfide bonds has shown that ERp57 is directly bound to the nucleic acid. Therefore our results demonstrate not only the nuclear presence of the protein in vivo, but also its interaction with DNA, which was previously shown to occur in vitro.

A further proof for the nuclear localization of ERp57 was provided by the immunofluorescence experiments performed on HeLa cells. Although the nuclear signal was rather low, as expected, the intracellular distribution of the protein was entirely different from that displayed by PDI, which showed an exclusive localization in the endoplasmic reticulum. In 3T3 cells, and in other cell lines as well, nuclear ERp57 could not be clearly detected by immunofluorescence. As mentioned before, a great difference in the amount of protein in the endoplasmic reticulum and the nucleus renders the immunofluorescence approach unpracticable, while the Western blotting of the cross-linked proteins or of the proteins from the internal nuclear matrix offers greater sensitivity and selectivity, and thus may still reveal the presence of a nuclear protein.

The reasons for an increased nuclear import of the protein in HeLa and other types of cells (e.g., chicken fibroblasts [Altieri et al., 1993]) are at present unknown.

Subnuclear localization and DNA-binding specificity of the mammalian enzyme are identical to those of avian enzyme. ERp57 has been found in the internal nuclear matrix preparations from 3T3 and HeLa cells. The enzyme purified from pig liver has been shown by gel shift experiments to recognize double-stranded DNA fragments with typical S/MAR features. Also with the mammalian enzyme, as with the avian one [Ferraro et al., 1999], a reducing treatment with DTT abolished the DNA-binding properties.

Thus it seems that these S/MAR-binding properties and the nuclear matrix localization are general features of ERp57, at least in a variety of higher vertebrates. It would be tempting to attribute to this protein a loop-anchoring function just like that of other identified nuclear matrix proteins. However, the small amount of nuclear ERp57 in mamalian cells argues against the hypothesis of a major structural role. Moreover the DNA sequences to which the protein is bound in vivo are still unknown, and on the other hand, the nuclear matrix is known to recruit many proteins with more specific functions, as for example transcriptions factors [Stein et al., 1991].

A hypothesis can only be made at present on the role of nuclear ERp57. It might be related to the redox activity of the thioredoxin-like sites, which are expected to be modulated by the binding to DNA, considering that just the oxidized form is bound. Also its chaperone activity might be relevant, since it has been pointed out [Roti Roti et al., 1998] that the proteins of the nuclear matrix are particularly susceptible to heat inactivation.

However, considering its propensity to bind calreticulin and the activated form of STAT3, which is imported in the nucleus, it is legitimate to hypothesize that ERp57 exerts its function in the nucleus at least in part by associating with these proteins. In particular, it is conceivable that ERp57 might enhance the nuclear import and/or modulate the DNA-binding of STAT3, while associating with calreticulin it might have a role in the recently discovered nuclear export function of this protein.

The way in which ERp57 (possibly associated with other proteins) is imported in the nucleus should be considered. Its C-terminal sequence QEDL is similar to the signal for retention in the endoplasmic reticulum. However this sequence differs from the canonical KEDL signal, and in fact the presence of ERp57 in cytosol has been demonstrated [Lewis et al., 1986; Ndubuisi et al., 1999]. The nuclear import should take place from the cytosol by means of

the nuclear localization signal PKKKKKA, also present in the C-terminal domain. Although the canonical sequence for the nuclear import is now considered to be a bipartite one [Dingwall and Laskey, 1991], this is absent from many nuclear proteins. The nuclear import signal of SV40 T antigen is represented by a sequence similar to that shown above and cristallographic studies have demonstrated that this sequence binds with high affinity to a specific site of importin, which is responsible for the nuclear import process [Dingwall and Laskey, 1998]. This might explain not only the nuclear localization of ERp57, but also that of the other ERp57-associated proteins.

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