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Intranuclear trafficking of plasmid DNA is mediated by nuclear polymeric proteins lamins and actin

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Functions of nuclear polymeric proteins such as lamin A/C and actin in transport of plasmid DNA were studied. The results show that the lamina plays an important role in plasmid DNA's entry into the cell nucleus from the cytoplasm. Selective disruption of lamin A/C led to a halt in plasmid DNA transport through the nuclear envelope. Inside the nucleus, plasmid DNA was frequently localized at sites with impaired genome integrity, such as DNA double-strand breaks (DSBs), occurring spontaneously or induced by ionizing radiation. Polymeric actin obviously participates in nuclear transport of plasmid DNA, since inhibition of actin polymerization by latrunculin B disturbed plasmid transport inside the cell nucleus. In addition, precluding of actin polymerization inhibited plasmid co-localization with newly induced DSBs. These findings indicate the crucial role of polymeric actin in intranuclear plasmid transport.

Keywords: plasmid DNA, DNA double-strand breaks, nuclear actin, lamin A/C

INTRODUCTION

Although genomic DNA is locally constrained, showing only very limited diffusion in interphase nuclei of living cells (Chubb *et al.*, 2002), little is known about the dynamics and localization of small circular DNA molecules that invade cells as a result of virus infection, during gene therapy, or during experimental cell transfection (Molas *et al.*, 2003). When a foreign DNA was introduced into a cell by transfection, it must cross the plasma membrane, move through the cytoplasm using actin and microtubule networks (Ondrej *et al.*, 2007), enter the nucleus, and then locate to a specific site appropriate for its integration and expression. Once foreign DNA has arrived at the nuclear envelope, it must either wait until the cell divides or be specifically imported through the nuclear pore complex to the nucleoplasm as was performed using exogenous DNA binding with NLS (nuclear localization sequences) containing peptide (Munkonge *et al.*, 2003). What happens to plasmid DNA after it has entered the nucleus, which remains an open question. It has been found that the movement of incoming DNA fragments of various sizes is more severely restricted in the nucleus than in the cytoplasm and, in contrast to similarly sized dextran molecules, DNA fragments do not diffuse freely in the nucleus (Lukacs *et al.*, 2000; Mearini *et al.*, 2004). The experiments of Meriani *et al.* (2004) show that plasmid DNA is not present in a soluble, freely diffusible form in the nucleus, indicating that it is bound to nuclear structures. It follows from their results that plasmids are bound to nuclear substructures and networks, such as nuclear matrices or scaffolds, which constrain their mobility.

The nuclear envelope is a specialized domain that forms the boundary of the eukaryotic cell nucleus. It consists of the inner and outer nuclear membranes, the nuclear pore complexes (NPCs) and the

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Abbreviation: DSB, DNA double-strand break; FCS, foetal calf serum; NLS, nuclear localization signal; PBS, phosphate-buffered saline.

lamina. The nuclear lamina is a meshwork of mixed intermediated filaments that are assembled from three lamins — A, B and C — forming a molecular interface between the inner nuclear membrane and chromatin (Shumaker *et al.*, 2003). Therefore, we focused on the role of the lamina in plasmid DNA's entry into the cell nucleus and its trafficking at the nuclear periphery.

Actin is another nuclear polymeric protein whose structure and function in the nucleus are not yet clearly understood, but the results of several observations show that it participates in many, if not all, nuclear processes. Nuclear actin is engaged in processing and transporting RNA, associates with small nuclear ribonucleoproteins (snRNPs) which have a major role in mRNA processing, and takes part directly in nuclear export of retroviral RNA (HIV-mRNA) (for a review see Bettinger *et al.*, 2004). Actin also forms complexes with RNA polymerase I and II (Hofmann *et al.*, 2001; 2004). In addition, nuclear actin has been found in association with chromatin remodelling and histone acetyltransferase complexes (for a review see Bettinger *et al.*, 2004). Together with emerin and nesprins, short actin filaments bind lamin A which forms the nuclear lamina with other lamins. It could represent links between the nuclear periphery and other subnuclear domains and chromatin (Shumaker *et al.*, 2003; Bettinger *et al.*, 2004; Holaska *et al.*, 2004; Libotte *et al.*, 2005).

Miller *et al.* (2003) and Porteus *et al.* (2003) found that induction of a double-strand break (DSB) in the target gene can increase the frequency of foreign DNA integration at that site. Foreign DNA's utilization of DSBs to integrate into the host genome represents risks for viral infection of the cell or damage to genetic information at the site of insertion. Thus DSBs are hazardous, not only due to the fact that they interrupt genetic information that if not repaired could cause the death of the cell or cause cancer to develop if it is misrepaired, but also because of the possibility for integration of infectious viruses into such sites. On the other hand, the ability of foreign DNA to locate into sites where both chains of DNA are interrupted and to integrate into the host genome could be favourable for targeting of advantageous genetic information to specific loci containing DSBs during gene therapy (Porteus *et al.*, 2003).

In this work, we investigated the role of lamin A/C in plasmid DNA's entry into the nucleus and the role of nuclear actin in trafficking of labelled plasmid DNA inside the nucleus. We also studied the possible role of nuclear actin in plasmid DNA transport to DSBs induced by γ-rays. We found that the plasmid DNA frequently co-localized with DSBs induced by irradiation and were also frequently localized very close to intranuclear actin aggregates. Inhibiting actin polymerization led to cessation of plasmid trafficking to DSBs. The results also demonstrated that lamin A/C is essential for the cytoplasm-nucleoplasm transport of plasmid DNA.

MATERIAL AND METHODS

Cell culture and cell transfection. Human lung fibroblasts and MCF-7 cells were grown in DMEM medium supplemented with 10% foetal calf serum (FCS), penicillin (100 U/ml) and streptomycin (100 μg/ml) in humidified air with 5% CO_2 at 37°C.

Silencing of the lamin A/C gene in MCF-7 cells was done using a kit (Santa Cruz Biotechnology) containing gene-specific siRNA and a transfection reagent. Transfection of cells with siRNA was performed according to the manufacturer's protocols in serum-free medium. After 5 h of cell transfection, the medium was replaced with DMEM containing 10% FCS and antibiotics, and cells were incubated for 48 h before evaluation of transfection efficiency.

To detect the role of A/C lamin in transport of plasmid DNA into the nucleus, labelled plasmid DNA was transfected into MCF-7 cells 24 h after beginning of siRNA transfection procedure.

Co-transfection of labelled plasmid DNA and vector for actin-Lumio fusion protein was performed in human fibroblasts. Transfections of the both cell types were done in OPTIMEM medium (Gibco) using Lipofectamine 2000 (Invitrogen) according to the manufacturer's protocol. After 5 h of cell transfection, the medium was replaced with DMEM containing 10% FCS without antibiotics, and cells were incubated for a further 24 h and fixed. Before fixation of the fibroblasts co-transfected with vector bearing actin-Lumio fusion gene, the expressed actin-Lumio protein was stained. To this end the DMEM was replaced with OPTIMEM supplemented with green Lumio reagent to obtain the recommended concentration of 2.5 μM. After that the fibroblasts were incubated for 30 min and then fixed, or they were irradiated before fixation.

Vectors and DNA labelling. The Lumio technology (Invitrogen) and the hORF clone of the β-actin gene (IOH3654, Invitrogen) were used for fusion protein preparation, where the fluorescent tag is a Lumio-tetracysteine sequence (green or red, depending upon the Lumio reagents used).

The plasmid DNA, composed of the pENTR 221 vector containing β-actin cDNA, the gene for kanamycin resistance, the Kozak consensus sequence and the pUC origin, was labelled before transfection with Label IT Tracker rea 0.4=gent containing Cy5 and/or Cy3 (Mirus Co.). Plasmid labelling was performed at 37°C for 1 h at the ratio of Cy5 (Cy3)

and DNA recommended by the manufacturer. The unbound Cy5 (Cy3) reagent was removed by DNA precipitation.

Drug treatment and DSB induction. Twenty-two hours after the beginning of fibroblast transfection with plasmid DNA, the cells were treated with the inhibitor of actin polymerization latrunculin B (20 μM) (Sigma-Aldrich) for 2 h. The treatment was carried out before cell fixation or irradiation. The DMEM medium containing latrunculin B was removed, the fibroblasts were washed three times with DMEM without latrunculin B and irradiated in this medium with a dose of 1 Gy of ^{60}Co γ-rays at a dose rate of 1 Gy/min from a distance of 60 cm and fixed at intervals of 30 and 120 min after irradiation. Thirty minutes before fixation at each time interval, the staining of actin-Lumio protein was performed.

RT-PCR analysis. The efficiency of A/C lamin gene silencing using siRNA was performed by RT-PCR analysis on control and siRNA-transfected MCF-7 cells 48 h after beginning of transfection. The MCF-7 cells (5×10^5) were trypsinized, washed 2 × in phosphate-buffered saline (PBS), pelleted and lysed with 200 μl of lysis buffer from the mRNA Capture Kit (Roche Applied Science). The poly(A^+) tail of mRNA liberated by cell lysis serves as a hybridization tag for biotin-labelled oligo$(dT)_{20}$, priming the reverse transcriptase reaction after washing off the lysate supernatant. Another function of oligo(dT) is to capture poly(A^+) RNA in streptavidin-coated PCR tubes. After immobilization of poly(A^+) RNA in streptavidin-coated PCR tubes, the solution was removed from the tube and the tube was washed three times with washing buffer from the kit. The captured mRNA was used for reverse transcription using the RT-PCR mix (Roche) to synthesize the first cDNA strand. After that, PCR reactions with specific primers for lamin A/C (Santa Cruz Biotechnology) were carried out.

Western blot analysis. Forty-eight hours after the beginning of siRNA transfection, cellular extracts of control and siRNA-transfected MCF-7 cells were obtained by lysis in 1% sodium dodecyl sulphate (SDS) buffer (1% SDS, 20% glycerol, 1 mM Tris, pH 7.5). Protein concentration was determined using DC Protein Assay Kit (Bio-Rad) according to the manufacturer's instructions. Equal amounts of total protein mixture from each sample were separated in 10% SDS/PAGE electrophoresis and electrotransferred onto a PVDF membrane (Amersham). Lamin A was immunodetected with a mouse anti-lamin A antibody (Chemicon International, Inc.). Finally, an anti-mouse secondary antibody conjugated with horseradish peroxidase was used. Protein bands on the membrane were detected using ECL+Plus reagent (Amersham). After immunodetection, each membrane was stained using amido black to confirm equal protein loading.

Cell fixation and immunostaining. Cells were fixed in 4% paraformaldehyde, washed in PBS, permeabilized in 0.2% Triton X-100 and blocked in 7% FCS + 2% bovine serum albumin (BSA/PBS). Primary antibodies from mouse were used for detecting actin (anti-actin clone C4 from Chemicon International, Inc.), lamin A (Chemicon International, Inc.) and γ-H2AX (Upstate Biotechnology, Inc.). Antibodies were diluted in 7% FCS + 2% BSA/PBS. Secondary antibodies were affinity-purified donkey anti-mouse-FITC-conjugated, anti-mouse-Cy3-conjugated, and anti-mouse-Cy5-conjugated from Jackson ImmunoResearch. Actin filaments were stained with rhodamine-phalloidin (Invitrogen). Chromatin was counterstained using 1 μM TO-PRO-3 (Molecular Probes) freshly diluted in 2 × saline sodium citrate (SSC). After brief washing in 2 × SSC, Vectashield medium (Vector Laboratories) was used for final mounting of slides.

Image acquisition and analysis of experimental data. An automated Leica DM RXA fluorescence microscope, equipped with a CSU10a Nipkow disc (Yokogawa, Japan) to produce a confocal effect, a CoolSnap HQ CCD-camera (Photometrix), and an Ar/Kr-laser (Inova 70C, Coherent), driven by a personal computer, were used for image acquisition (Kozubek *et al.*, 2001). Automated exposure, image quality control and other procedures were performed using FISH 2.0 software (Kozubek *et al.*, 2001). An oil immersion Plan Fluotar objective (100 ×/NA 1.3) was used. Forty optical sections at 0.2 μm steps along the z-axis were acquired for each nucleus, using the FISH 2.0 software, at a constant temperature of 26°C, and were stored in the computer memory.

Off-line image analysis of fluorescence signals was performed using the FISH 2.0 software (Kozubek *et al.*, 2001). Evaluation of data and statistical analyses were performed using the Sigma Plot statistical package (Jandel Scientific). Intensity profiles of R-G-B channels were obtained using Andor iQ 1.2.0 Software (Andor Technology).

RESULTS

Plasmid DNA entry to the cell nucleus

To investigate the function of the lamina in plasmid DNA cyto-nucleoplasmic transport, we silenced expression of lamin A/C in MCF-7 cells using specific siRNA. MCF-7 cells were used in the experiments because of the high efficiency of siRNA transfection into these cells, as opposed to fibroblasts. Forty-eight hours after the beginning of siRNA

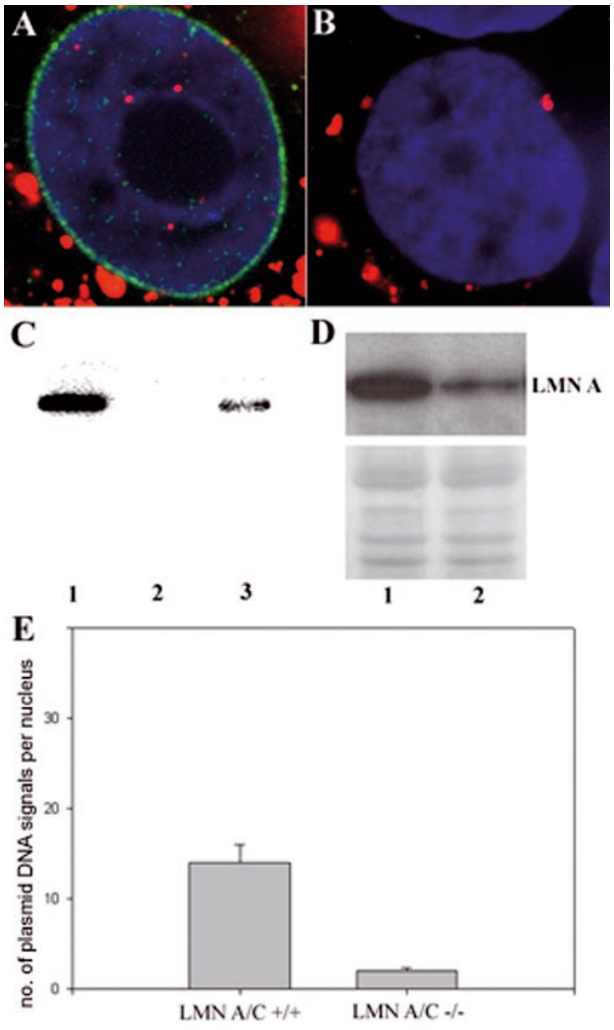

Figure 1. Detection of lamin A/C in MCF-7 cells with silenced expression of lamin A/C gene and in control cells.
(A) Nucleus of a control cell transfected with plasmid DNA labelled by Cy3 (red) after immunodetection of lamin A (green). Chromatin is counterstained with TO-PRO-3 (blue). Plasmid DNA can be seen in the cytoplasm but also inside the nucleus. (B) Nucleus of a cell with inhibited expression of lamin A/C. In contrast to the control nucleus, lamin A/C is missing and plasmid DNA remains only in cytoplasm. (C) Comparison of lamin A/C expression in cells treated with siRNA and in control cells using RT-PCR of 383 bp fragment of lamin A/C gene. Lane 1: 400 bp part of DNA ladder. Lanes 2 and 3, respectively: lamin A/C in silenced and in control cells. (D) Western blot analysis using anti-lamin A. Lane 1: non-transfected control cells. Lane 2: lamin A/C silenced cells. Total protein (10 μg) for lamin A (70 kDa) detection was loaded onto 10% PAGE. (E) Number of plasmid DNA signals per nucleus of control and lamin A/C silenced cells (n = 60).

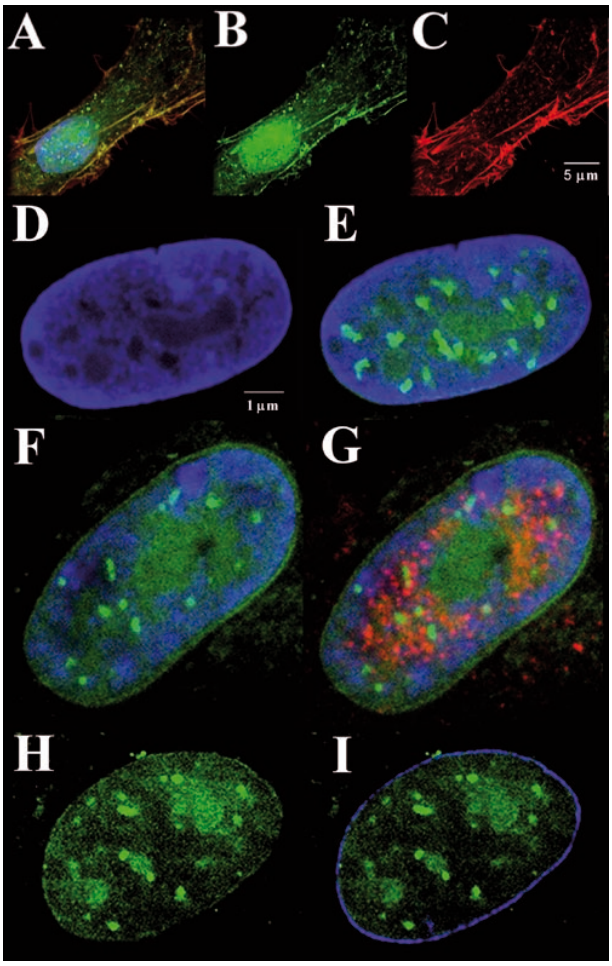

Figure 2. Central XY optical slices through nuclei of fibroblasts, showing structures formed by β-actin.
(A) Fibroblast transfected with vector bearing gene coding for the actin-Lumio fusion protein. It formed cytoplasmic actin filaments, visualized in green (B), which strongly co-localized with phalloidin staining (red) as was documented in (Ondrej *et al.*, 2007) (C). (D) Chromatin of a fibroblast nucleus stained with TO-PRO-3 (blue). (E) The same nucleus as in (D), showing the structure and location of β-actin-Lumio (green) in relation to the density of chromatin stained by TO-PRO-3 (blue). (F) Nucleus expressing β-actin-Lumio protein (green) was immunostained (G) with anti-β-actin antibody (red). It can be seen that the β-actin-Lumio located in heterochromatin and at the periphery of the nucleus is not labelled by the antibody. (H) The β-actin-Lumio fusion protein also formed a perinuclear actin shell. (I) This shell co-localized with the lamina, immunostained with anti-lamin A antibody (blue).

transfection, the inhibition of target gene expression reached a maximal level. Twenty-four hours before the maximal lamin A/C expression inhibition, the MCF-7 cells were transfected with labelled plasmid DNA. The decrease of lamin A/C expression was revealed by RT-PCR and Western blotting (Fig. 1C, D) forty-eight hours after siRNA transfection. The bright rim around the cell nucleus detected by fluorescence of anti-lamin A antibody in control cells (Fig. 1A) was weak or completely disappeared in approx. 80% of cells (n = 60) forty-eight hours after their transfection with siRNA (Fig. 1B). In these cells, plasmid DNA was mostly arrested in the cytoplasm around the cell nucleus, while in control cells plasmids were

commonly localized inside the cell nucleus (Fig. 1). The average number of plasmid molecules localized inside the cell nucleus significantly decreased in cells with disrupted lamin expression (Fig. 1E). This indicates an important function of the lamina in plasmid DNA transport from the cytoplasm into the nucleus.

Nuclear actin

The β-actin-Lumio fusion protein formed cytoplasmic actin filaments that were simultaneously stained in a standard way with phalloidin (Fig. 2A–C) during its transient expression to confirm that Lumio really stains the β-actin. Variously shaped structures of β-actin-Lumio were detected also inside the fibroblast nucleus, but these structures were not labelled by phalloidin. The nuclear structures were circular, elongated and tube-like rods on the border between more and less compact chromatin; amorphous structures that filled the nuclear space that was faintly labelled by TO-PRO-3 (Fig. 2D, E); and, in some nuclei, also the perinuclear shell (Fig. 2F, H). The β-actin-Lumio fusion protein slightly co-localized with the antibody against β-actin (Fig. 2F, G). The antibody co-localized with β-actin-Lumio mainly in euchromatin (faintly labelled by TO-PRO-3), but it did not label the perinuclear shell and did not give the same image of actin structure as did β-actin-Lumio. While β-actin-Lumio makes it possible to see actin in various structural forms, the antibody only provides information about the presence of actin but not about its detailed structure. Differently shaped aggregates of actin visualized using the fusion protein are not clearly distinguished using the antibody. The fusion protein (β-actin-Lumio) also allowed visualization of the perinuclear actin shell, which is closely associated with the inner part of the lamina detected with anti-lamin A antibody (Fig. 2H, I).

The plasmid DNA bound to cytoplasmic actin filaments with high frequency (our unpublished results). When plasmid DNA passed into the nucleus, it also bound to the perinuclear actin shell and to intranuclear actin aggregates. About 40% of intranuclear plasmid DNA signals were localized very close to actin aggregates (Fig. 3, compare blue — DNA and green — actin).

DSBs as a destination for plasmid DNA

DSBs were induced by γ-irradiation with a dose of 1 Gy in fibroblasts 24 h after their co-trans-

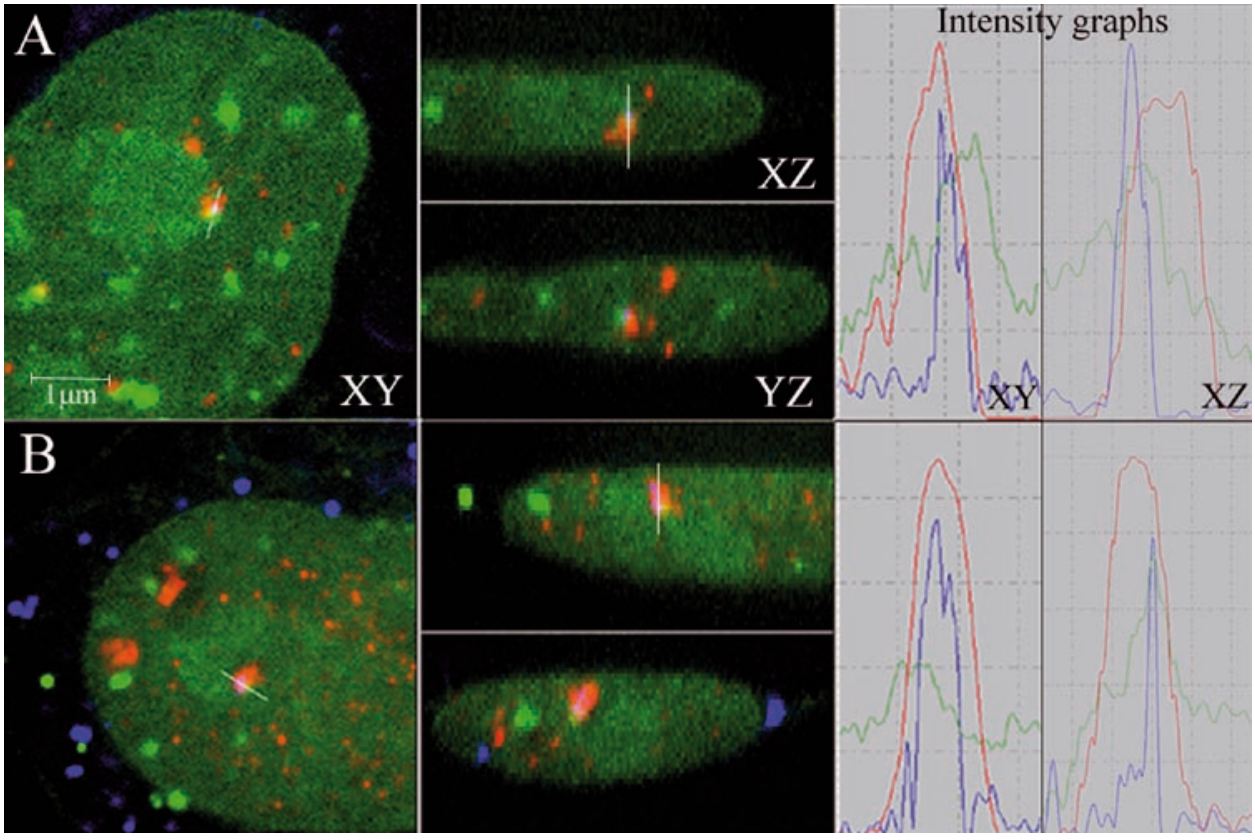

Figure 3. Co-localization of plasmid DNA (blue) with actin structures (green) and DSBs (red) within the nucleus. (A) γ-H2AX (red) in a non-irradiated cell, (B) γ-H2AX (red) in a cell 2 h after irradiation. Optical slices through one selected signal of plasmid DNA (blue) on the XY, XZ and YZ axes are presented at right. Graphs show the intensity of blue, red and green colour measured along the white line on the XY and XZ slices.

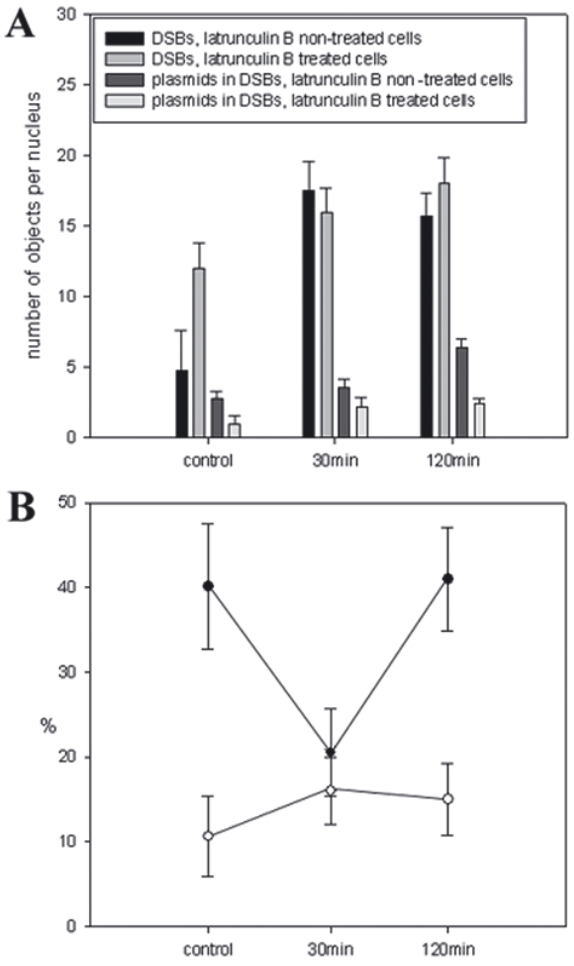

Figure 4. Co-localization of plasmid DNA and DSBs at various times after γ-irradiation of human fibroblasts (no. of cells = 20 per each type of experiments).
(A) Mean numbers per nucleus of DSBs and of plasmid DNA molecules associated with DSBs, detected at different times PI in latrunculin B-treated and untreated cells irradiated with a dose of 1 Gy. (B) The percentage of DSBs co-localizing with plasmids in untreated cells (filled points) and latrunculin B-treated cells (open points). The bars indicate standard deviation.

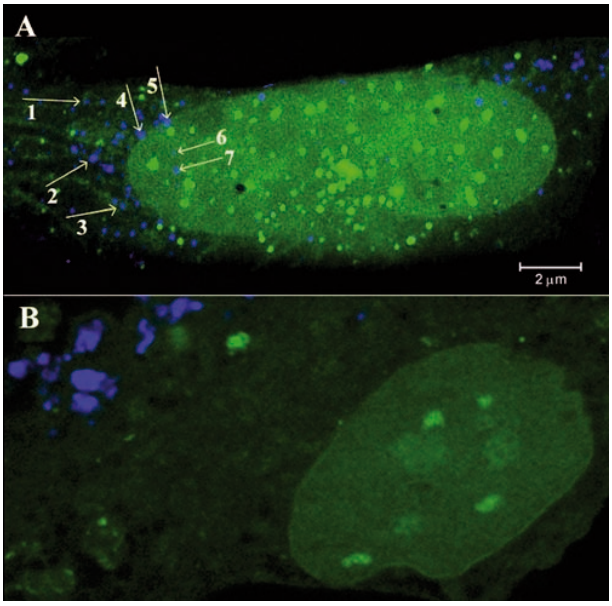

Figure 5. Projection of maximal images of fibroblasts transfected with the β-actin-Lumio (green) gene.
(A) Image of a fibroblast, showing co-localization of plasmid DNA (blue) with cytoplasmic actin filaments (green) (arrows 1–3), with perinuclear actin (arrows 4–5), and plasmids localized inside the nucleus (arrows 6–7) (see also Ondrej *et al.*, 2007). (B) Image of a fibroblast after inhibition of actin polymerization using latrunculin B. The cytoplasmic actin filaments have disappeared, the shape of the nucleus has changed, and the number of intranuclear actin aggregates decreased. Plasmid DNA (blue) accumulates as large aggregates at the periphery of the cytoplasm.

fection with labelled plasmid DNA and vector bearing cDNA encoding the actin-Lumio fusion protein. The irradiated fibroblasts were fixed 30 or 120 min post irradiation (PI). DSBs induced by γ-irradiation or during the normal cellular processes were detected using an antibody against phosphorylated H2AX (γ-H2AX) (Fig. 3A, B). In non-irradiated cells (fixed 24 h after co-transfection with labelled plasmid DNA and actin-Lumio vector), the number of DSBs was low. This number was significantly higher 30 min PI and decreased slightly during 120 min after irradiation (Fig. 4A). At this time, the number of actin aggregates per nucleus was significantly higher than in non-irradiated control cells (not shown). After irradiation, the number of plasmid molecules located in DSBs increased to reach its maximum 120 min PI (Fig. 4A). The frequency of co-localization of plasmid DNA with DSBs is shown in Fig. 4B in relation to PI time. Under standard experimental conditions, nearly 35% of DSBs in non-irradiated cells co-localized with plasmid DNA (Fig. 4B), showing that DSBs were the preferred destinations for plasmids. After irradiation, the percentage of DSBs co-localizing with plasmid DNA decreases significantly due to the increased number of DSBs. Two hours later, the percentage of co-localization reached the same level observed in non-irradiated cells, but with a substantially higher number of DSBs, indicating a higher number of plasmid molecules co-localizing with DSBs.

The application of an actin polymerization inhibitor, latrunculin B, dramatically changed actin distribution inside the nuclei and disrupted cytoplasmic actin networks (Fig. 5). Not only was the shape of the nuclei changed, but also the size and the number of nuclear actin aggregates decreased by more than 50% (not shown). Non-irradiated but latrunculin B-treated cells showed a significantly higher number of DSBs per nucleus than did cells not treated with the actin polymerization inhibitor

(Fig. 4A). After irradiation of latrunculin B-treated cells, the number of DSBs did not decrease 2 h PI but increased. Non-irradiated cells treated with latrunculin B also showed a significantly lower number of plasmids associated with DSBs than untreated cells; the number of plasmid DNA co-localizing with DSB remained at the same level until the end of observation (120 min PI), showing a significant difference in comparison with irradiated cells not treated with the actin inhibitor (Fig. 4).

DISCUSSION

The data presented here demonstrate the importance of the lamina in the cyto-nucleoplasmic transport and actin in the intranuclear transport of plasmid DNA. Despite the barrier provided by the nuclear envelope, viruses infect non-dividing cells and also plasmids have been delivered into cells that do not divide. The results of several authors (Dowty *et al.*, 1995; Dean, 1997; Munkonge *et al.*, 2003) suggest that exogenous DNA bound with proteins containing NLS or DNA itself carries some nuclear recognition signals utilizing nuclear pores. The nuclear envelope consists of the inner and outer nuclear membranes, the lamina, and nuclear pore complexes (reviewed in Shumaker *et al.*, 2003; Mattaj, 2004). It has been suggested that the nuclear lamina is involved in maintaining the structural integrity of the nuclear envelope and influences chromatin structure and function. It is also tightly associated with specific nucleoporins that are constituents of the nuclear pore complexes (Smythe *et al.*, 2000; Hawryluk-Gara *et al.*, 2005). Thus, disruption of the lamina in our experiments could negatively affect the function of nuclear pores and arrest plasmid DNA transport from the cytoplasm to nucleoplasm, as we indeed observed. Lamina is a meshwork of lamins, actin (Fig. 2H, I) and other lamin-binding proteins (reviewed in Shumaker *et al.*, 2003) that links the nuclear envelope, chromatin, DNA replication complexes and barrier-to-autointegration factor complexes. We suggest that the lamina also represents an essential platform for exogenous DNA transport from the nuclear periphery into more central compartments of the cell nucleus.

The actin visualized with the Lumio tag had several structural forms. In the cytoplasm, it formed long filaments along the longer side of the cell which disappeared after the inhibition of actin polymerization (Figs. 2A–C and Fig. 5). A similar observation was described by Münter *et al.* (2006). At the nuclear periphery, it formed a thin perinuclear layer which co-localized with the lamina (Fig. 2H, I). This actin layer was not visible in all cells (it was visible in approx. 40% of cell nuclei). It is probable that the perinuclear actin shell is present only in just-divided cells, as described by Clubb and Locke (1998). We observed that, inside the nucleus, actin was organized into short rods, as described by a number of investigators (for a review, see Pederson & Aebi, 2005), and it also diffusely filled the inner part of the nucleus that was faintly stained with TO-PRO-3 (euchromatin) (Fig. 2).

We observed that on its way to the nucleus plasmid DNA binds to the cytoplasmic actin filaments (Fig. 5A). In untreated cells, numerous plasmid molecules accumulated in the cytoplasm around the nuclear envelope, in contact with actin, and some were just passing through the perinuclear actin shell (Fig. 5A). After inhibition of actin polymerization, plasmid DNA located inside the cytoplasm accumulated in huge aggregates at the cellular periphery (Fig. 5B); although there were some plasmids in the nucleus, they probably had reached it before actin was depolymerized.

In the cell nucleus, plasmids were most frequently located at specific sites characterized by transient interruption of DNA integrity. It seems that plasmids move to find those sites where the integrity of the genome is impaired and which probably facilitate their integration. Very often, plasmids were co-localized with DSBs detected with an antibody against γ-H2AX in γ-irradiated or non-irradiated cells, where DSBs arise as a result of various nuclear processes (Foster & Downs, 2005).

The use of DSBs induced by *Sce*I endonuclease as a target for adenovirus vector integration into the genome has been described by Miller *et al.* (2003) and Porteus *et al.* (2003). We also observed DSB-targeting of foreign DNA. The number of plasmid molecules co-localizing with DSBs increases during the time after irradiation. However, inhibition of actin polymerization stopped this process, and the number of plasmid molecules co-localizing with DSBs remained low (similar to the number in non-irradiated cells treated with the actin inhibitor). On the basis of this result, we suppose that the low level of plasmid co-localization with DSBs during the time after irradiation is a consequence of the disruption of the cytoplasmic and nuclear actin skeletons.

The nuclear trafficking of plasmid DNA is still poorly explored, even though targeting and delivery of genetic material is a highly important problem in gene therapy. As reviewed by Verkman (2002), molecules of DNA larger than 2000 bp are unable to diffuse freely in the cytoplasm because of their binding to actin filaments. The role of β-actin in many nuclear processes, such as RNA transcription, processing and export, has already been described (Hofmann *et al.*, 2004). β-Actin has also been identified as a component of chromatin remodelling complexes (for a review, see Bettinger *et al.*, 2004). Some authors

(Lukacs *et al.*, 2000; Mearini *et al.*, 2004) followed the dynamics of microinjected DNA inside the cell nucleus, and, because of the plasmids' low diffusive movement compared with dextran molecules of the same size, they deduced that those probably bound to some intranuclear structures or components. Our recent results (Ondrej *et al.*, 2006) show that plasmid DNA additionally displays non-random directional movement in the nucleus, indicating that there must exist some mechanism immobilizing the plasmid and driving its movement towards specific sites inside the nucleus. The directional movement of plasmid molecules inside the nucleus (Ondrej *et al.*, 2006), their binding to the cytoplasmic actin network (Verkman, 2002), and the presence of nuclear actin in forms varying from monomers to polymers (McDonald *et al.*, 2006) forming a nucleoskeletal structure in *Xenopus* egg nucleus (Kisileva *et al.*, 2004) suggests that nuclear plasmids are also attached to actin polymers and could move along them for short distances. The role of polymeric actin in plasmid mobility is demonstrated in our work by the cessation of plasmid movement in cells treated with a substance inhibiting actin polymerization (latrunculin B), leading to low occupation of DSBs by plasmids.

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