SUPPLEMENTARY DATA

LEGENDS TO FIGURES

Supplementary Fig. 1:

The c.a. inclusions are devoid of viral nucleic acids, splicing components and capsid proteins.

EM in situ hybridization of viral DNA (**A**) and host snRNA (**B**). The c.a. inclusions (stars) are devoid of gold particles, whereas virus particles (*v*), the clusters of interchromatin granules (*ig*) and the fibrillogranular (*fg*) network are labeled.

I-EM labeling of viral hexon (**C**) and penton-base (**D**) proteins, using rabbit polyclonal anti hexon (Puvion-Dutilleul *et al*.,1999) and anti-penton base (Rosa-Calatrava *et al*., 2001) antibodies. The c.a. inclusions (stars) are very poorly or not labeled, respectively, whereas protein crystals (*pc*) and viruses (*v*) are strongly stained. *c*: cytoplasm; *ch*: chromatin; *e*: electron transluscent area; *oi*: IVa2- induced electron opaque inclusion.

All steps of Ad5-induced remodeling of the host nuclear ultrastructure were described in Puvion-Dutilleul *et al*. (1994, 1995, 1999).

Supplementary Fig. 2:

The coiled-coil domain of pIX is involved in the formation and the colocalisation of pIX inclusions with ND10 at the nuclear matrix.

(**A**) Colocalization with PML bodies. A549 cells were transfected with plasmids expressing pIX wt (a) or a set of mutant pIX (b-d), and analyzed by IF-staining, using monoclonal anti-PML (PMG3) and polyclonal anti-pIX antibodies . Bars, 10 μm.

(**B**) Association with the host nuclear matrix. Cytosolic (*c*), nucleoplasmic (*n*), chromatin-associated (*ch*) extracts and nuclear matrix (*m*) fractions were prepared (Dutertre *et al*., 2000) from A549 cells,after transfection with the indicated pIX expressing plasmids (lanes 1-16) or infection (28 h pi) with Ad5 wt or Ad5 (IX/V117D) (lanes 17-24). Equal amounts of each fraction (normalized from whole cell extracts) were analyzed by immuno-blotting with polyclonal antibodies against pIX.

(**C**) *In situ* nuclear matrix (NM) IF staining (Huang *et al*., 1998). A549 cells were transfected with plasmids expressing the pIX wt (a) or pIX (V117D) (b) and analysed by IF staining as in (A). Bars, 10 μm.

(**D**) The formation of pIX-induced inclusions interferes with As-mediated disruption of ND10 bodies. A549 cells were transfected with plasmids expressing wt (a-c) or a set of mutant pIX variants (d-i) and, 12 h later, treated for 36 h with 1 μM As. Cells were analysed by IF staining as in (A). Panels c, f and i are the merged confocal images obtained with each fluorochrome separately from a/b, d/e and g/h, respectively. ND10 were persistent after As treatment, only in the presence of pIX mutants which retain the ability to induce inclusions. Bars, 10 μm.

Conclusions:

Altogether, our findings (A-C) clearly indicate that processes, which direct the formation and nuclear matrix retention of the pIX inclusions, generally in close connection with PML bodies, are specifically linked and dependent on the integrity of the coiled-coil domain of pIX. They suggest that PML bodies and/or their corresponding nuclear matrix anchoring sites constitute privileged nucleation sites for the formation of pIX inclusions.

Supplementary Fig. 3:

Physical characteristics of Ad5 wt and Ad (IX/V117D) viruses.

(**A**) Titration of the viruses produced on 293 cells. Cells ($3x10^8$) were infected with Ad5 wt or Ad (IX/V117D) viruses at 3 p/cell. At 48-72 h pi, the cells were harvested and viruses were extracted, purified and titer (IU/ml) determined. The titer of Ad5 (IX/V117D) is 6-fold reduced compared to that of Ad5 wt. However, the two viruses showed similar particle/IU (p/IU) ratios. The results are representative of three independent productions.

(**B**) Similar infectivities of Ad5 wt and Ad5 (IX/V117D) on 293 cells. Cells were infected as indicated above. At 12 h pi, the cells were immuno-stained with anti-DBP antibodies . Bar, 15 μm.

(**C**) Thermostabilities of viruses. Aliquots of Ad5 wt, Ad5 (IX/V117D) and Ad5 dl313 (Colby *et al*., 1981) were incubated at 42°C for different time intervals and then titrated on 293 cells. Average values (with error bars) of triplicate experiments are represented. Ad5 wt and Ad5 (IX/V117D) have similar thermostabilities.

SPECULATIONS

Ad is being used as a vector for the efficient delivery of genes of therapeutic interest into cultured cells or in patients during clinical trials. In this latter context, the success of most Ad vectors depends both on the efficient transgene expression and the absence of vector-induced toxicity.

It has been proposed that PML bodies take part in cellular anti-viral processes by driving the retention and degradation of foreign viral material (Fabunmi *et al*.,

2001, Lallemand-Breitenbach *et al*., 2001). As in the case of Ad5 wt infection (Doucas *et al*., 1996, Ishov *et al*., 1996), the genomes of replication-defective Ad vectors preferentially accumulate next to PML bodies (our unpublished data). If, as suggested, this process affects transgene expression, this would be particularly sensible in the case of replication-defective vectors where PML bodies are not neutralized, due to the absence of both E4orf3 and pIX expression. In this background, the introduction of a pIX expression cassette, potentially counteracting ND10-associated antiviral-activities, should be advantageous. It will be of interest to compare the efficiency of transgene expression from such a vector with that of second generation vectors (AdΔE4) expressing E4orf3 product (Lusky *et al*., 1999), to determine which of ND10 disruption or sequestration is most beneficial.

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