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T-Antigen Interactions With Chromatin and p53 During the Cell Cycle in Extracts From Xenopus Eggs

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Abstract The role of SV40 large tumor T-antigen in replication of viral DNA is well established, but it is still unclear how T-antigen triggers cellular replication and cell transformation in non-permissive cells. Here, we used Xenopus egg extracts which reproduce most nuclear events linked to the cell cycle in vitro to analyze its interaction with genomic chromatin during the cell cycle. We show that T-antigen associates with chromatin before the nuclear membrane formation, and further demonstrate that the nuclear membrane is not necessary for its import into the nucleus. We show that the interaction of T-antigen with the endogenous chromatin does not occur at replication foci nor at RPA pre-replication centers. Immunoprecipitations as well as sucrose gradient experiments, indicate that the endogenous pool of p53 interacts with T-antigen. In addition, a transient association of both proteins with the nuclear matrix is observed during the ongoing DNA synthesis. These data are discussed in view of the T-antigen and p53 activity during the cell cycle. J. Cell. Biochem. 75:288–299, 1999.

Key words: Xenopus eggs; T-antigen; chromatin; nuclear matrix; nuclear import

The large T-antigen of the SV40 virus is a multi-functional protein involved in the control of transcription and replication of viral DNA, as well as in the transformation of host cells infected by the virus. T-antigen is a prototype of a replication initiator protein [for review see Fanning and Knippers, 1992]. T-antigen binds to the origin of replication of SV40 and promotes the formation of a pre-replication complex [Borowiec and Hurwitz, 1988; Dean et al., 1992; Deb and Tegtmeyer, 1987]. Extensive studies of the role of T-antigen on SV40 replication have demonstrated that the protein functions as a helicase and interacts with components of the cell replication machinery [Dornreiter et al., 1990, 1992; Fanning and Knippers, 1992; Smale and Tjian, 1986], such as the single-stranded DNA binding protein RPA and DNA polymerase-α [Dean et al., 1987; Kamakaka et al., 1994]. p53 and retinoblastoma protein Rb, two proteins encoded by tumor suppressor genes, can associate with the T-antigen and participate in the oncogenic property of this viral protein.

The oncogenic function of T-antigen that leads to immortalization of cells is poorly understood. It is generally believed that the oncogenic transformation is triggered through the p53 and pRB pathways [Moran, 1993; Perry and Levine, 1993]. However, a direct role of T-antigen in the regulation of DNA replication has been clearly established only for the viral genome. In vitro systems for DNA replication deriving from Xenopus eggs provide a convenient way to analyze the interaction of T-antigen with the genomic DNA during the cell cycle, and DNA replication in the virus-free context. Xenopus egg extracts reproduce most of the cell cycle events that occur in vivo, including chromatin decondensation, formation of a nuclear membrane, DNA replication, and mitosis [Blow and Laskey, 1986; Lohka and Matsui, 1983].

Here, we introduced T-antigen in a Xenopus cell-free extract and investigated its localization and interaction with genomic chromatin during nuclear formation and DNA replication. Although T-antigen does not interact either with the pre-replication foci, or with the replication foci, both immunofluorescence and biochemical data indicate distinct modifications in its local-

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ization that are linked to the progression in the cell cycle. An interaction with the endogenous maternal stock of p53, stored in the egg, was detected. Complexes between p53 and T-antigen are formed without affecting the rate of DNA replication in the extract. The association of p53 and T-antigen was also found to occur preferentially at the nuclear matrix.

MATERIALS AND METHODS

Xenopus Egg Extracts and T-Antigen

Interphase and mitotic Xenopus egg extracts were prepared as described elsewhere [Murray, 1991], except that the interphase extract buffer contained 0.25 mg/ml cycloheximide. Interphase high speed extracts were prepared by additional centrifugation of the interphase extract at 100,000*g* for 1.5 h at 4°C. The extracts were adjusted to 3% glycerol, aliquoted, frozen in liquid nitrogen, and stored at −80°C.

T-antigen was expressed in a baculovirus system and purified as described elsewhere [Hoss et al., 1990].

DNA Replication in Xenopus Extracts

Demembranated Xenopus sperm nuclei (2×10^5) were incubated in 100 µl Xenopus egg extracts containing 0.5 µg purified T-antigen and 7.5 µM Biotin-16-dUTP (or 20 µCi [^{32}P]-dCTP when indicated), for various times at 23°C. For immunofluorescence analysis, the reactions were stopped by addition of 200 µl of a fixation buffer (50 mM sucrose, 50 mM KCl, 5 mM $MgCl_2$, 0.5 mM EDTA, 4% formaldehyde, 20 mM HEPES pH 7.5).

DNA replication was quantitated by TCA precipitation of DNA extracted by proteinase K and phenol, followed by counting.

Analysis of T-Antigen Complexes in the Xenopus Cell Extract by Sucrose Gradient Sedimentation

Two-hundred µl Xenopus egg extract containing 0.5 µg purified T-antigen was incubated for 60 min at 23°C and then layered over a 5–20% sucrose gradient in a buffer containing 50 mM sucrose, 50 mM KCl, 5 mM $MgCl_2$, 0.5 mM EDTA, 20 mM HEPES pH 7.5, and centrifuged for 3 h at 55,000 rpm at 4°C in the Beckman SW55Ti rotor. The fractions were collected, run on an SDS-page, transferred onto nitrocellulose filters, and probed with antibodies against T-antigen and p53.

Isolation and Extraction of the Nuclear Matrix

Demembranated Xenopus sperm nuclei were incubated in Xenopus egg extracts at 23°C for 60 min. The nuclear matrix was prepared by digestion with 100 µg/ml DNase I for 30 min at 0°C followed by extraction with a solution containing 20 mM Tris, 10 mM EDTA, and 0.5, 1, or 2 M NaCl for 30 min at 0°C. The nuclear matrices were then analyzed by either SDS-PAGE and Western blotting, or immunofluorescent microscopy.

Immunofluorescent Microscopy

After incubation of the demembranated sperm nuclei in Xenopus extracts, the nuclei were diluted 10-fold with the fixation buffer. After 30 min incubation at room temperature, the nuclei were overlaid onto a 0.8 M sucrose cushion in the same buffer without formaldehyde, and spun onto glass cover slips. The cover slips were then incubated with mouse anti-T-antigen monoclonal antibodies [Puvion et al., 1988], or rabbit anti-p53 antibodies [Tchang et al., 1993], or Xenopus anti-RPA antibodies. The appropriate secondary antibodies, coupled with either FITC or Texas Red, were used in dilution of 1:100. Biotin-16-dUTP incorporation into DNA was detected with streptavidin conjugated with Texas Red. Samples were mounted with Citifluor (Citifluor Ltd., Cambridge) and examined under a BDS video microscope (BDS).

Immunoprecipitation

One-hundred µl of a Xenopus egg extract containing 0.5 µg purified T-antigen was incubated for 60 min at 23°C and then immunoprecipitated with either anti-p53 or T-antigen antibodies. The immunoprecipitated material was washed, proteins were then separated by SDS-PAGE, blotted onto a Hybond C+ membrane. Proteins present in the immunoprecipitate were revealed with the appropriate antibodies.

RESULTS

Dynamics of T-Antigen Localization in the Cell Cycle

When demembranated sperm nuclei are introduced into a low speed Xenopus egg extract, a sequence of morphological changes takes place that is characteristic of the nuclear events linked to the cell cycle [Blow and Laskey, 1986; Hutchison et al., 1988; Lohka and Matsui, 1983]. Decondensation of sperm chromatin is followed

by the assembly of the nuclear membrane in a step functionally corresponding to the G1 phase of the cell cycle. An S phase then occurs and the nuclei enter a G2-like phase.

We first prepared nuclei of monkey kidney CV-1 cells infected with SV40 and incubated them in Xenopus egg extracts. In these conditions, DNA replication of both cellular and SV40 DNA was detected (data not shown). However, it was not possible to distinguish between involvement of T-antigen in replication of either cellular or viral DNA using immunofluorescence. The incubation of demembranated sperm nuclei in Xenopus egg extracts supplemented with T-antigen was then used as a system to investigate how T-antigen interacts with cellular chromatin undergoing a synchronized cell cycle.

T-antigen localizes on chromatin as early as 5 min after the beginning of incubation, during chromatin decondensation (Fig. 1). A granular, dotted staining was observed both during formation of the nuclear membrane (15 min), and at later stages, when DNA replication began (see also Fig. 2A). At G2 phase, the distribution of T-antigen became diffuse and nucleolar staining appeared (Fig. 1, 120 min), as previously described in cells abortively infected with SV40 [Puvion et al., 1988]. We did not detect T-antigen staining in mitotic chromosomes.

The observed changes in the distribution of T-antigen in the nucleus during the cell cycle led us to investigate its distribution in relation to the localization of the replication foci and the proteins involved in DNA replication. DNA replication occurs in discrete replication foci, which can be detected by Biotin-dUTP incorporation. RPA, a single-strand DNA binding protein, binds to chromatin in the pre-replication foci that later become the sites of active DNA synthesis [Adachi and Laemmli, 1992]. During the rapid chromatin decondensation stage, a punctate staining of T-antigen can be observed prior to the appearance of RPA staining and Biotin-dUTP incorporation (Fig. 2A, 15 min). When DNA replication occurred, punctate RPA and BiodUTP staining was apparent, but there was no obvious colocalization of T-antigen and these foci (Fig. 2A, 60 min). G2 nuclei exhibited a uniform nuclear staining for both T-antigen and RPA localization (not shown). These observations indicate that T-antigen readily binds to chromatin, but does not colocalize preferentially with either the active sites of DNA synthesis or the pre-replication foci. No colocalization with PCNA, a marker for the elongation step of DNA replication, was observed in our experiments as well (data not shown).

The presence of T-antigen to the Xenopus egg extract did not affect either the rate or the kinetics of nuclear DNA synthesis, as monitored by measuring TCA-precipitable material (Fig. 2B) and by agarose gel electrophoresis (not shown). As DNA replication is very active in Xenopus egg extract, an additional effect of T-antigen might not be detected. However, this possibility did not exclude that T-antigen could interact with replication factors at replication centers. The absence of such interaction indicates that the binding of T-antigen to chromatin was not due to an association with replication machinery. No dominant negative effect of T-antigen on potential targets involved in DNA replication was detected.

Import of T-Antigen Into the Nucleus Does Not Require a Nuclear Membrane

An active and selective protein import occurs when the nuclear envelope is formed around chromatin [Newmeyer et al., 1986a,b]. The SV40 T-antigen contains a strong nuclear localization signal (NLS) [Kalderon et al., 1984; Lanford and Butel, 1984] that directs T-antigen to the nucleus via nuclear membrane transport mechanisms. However, we observed that T-antigen entered the nucleus at very early stages of incubation, before the nuclear membrane had been formed (Fig. 1).

In order to study the contribution of the nuclear membrane to the loading of T-antigen onto chromatin, we incubated demembranated sperm nuclei in a membrane-free high speed extract [Méchali and Harland, 1982; Sheehan et al., 1988]. Nuclear membrane reconstitution does not occur in these extracts and double-stranded DNA synthesis is not detected. In these conditions, we observed a pattern of T-antigen distribution within the nuclei that was similar to that observed in a low speed extract (Fig. 3). Although subtle changes in T-antigen distribution may not be detectable by immunofluorescence staining, this experiment demonstrates that the association of T-antigen to chromatin does not require that the membrane pore lamina complex is initially formed.

T-Antigen Interacts With the Endogenous p53 Stored in the Egg

The interaction of p53 with T-antigen is responsible for the inhibition of SV40 origin-dependent DNA replication in murine cells [Braithwaite et al., 1987]. p53 is present in large amounts in Xenopus eggs [Tchang et al., 1993], and we have recently observed that it enters the nuclei after the formation of the nuclear membrane [Amariglio et al., 1997; Tchang and Méchali, unpublished data], i.e., in a manner that differs from that of T-antigen.

We analyzed the localization of both SV40 T-antigen and p53 by using a monoclonal antibody directed against T-antigen and polyclonal affinity-purified antibodies against Xenopus p53

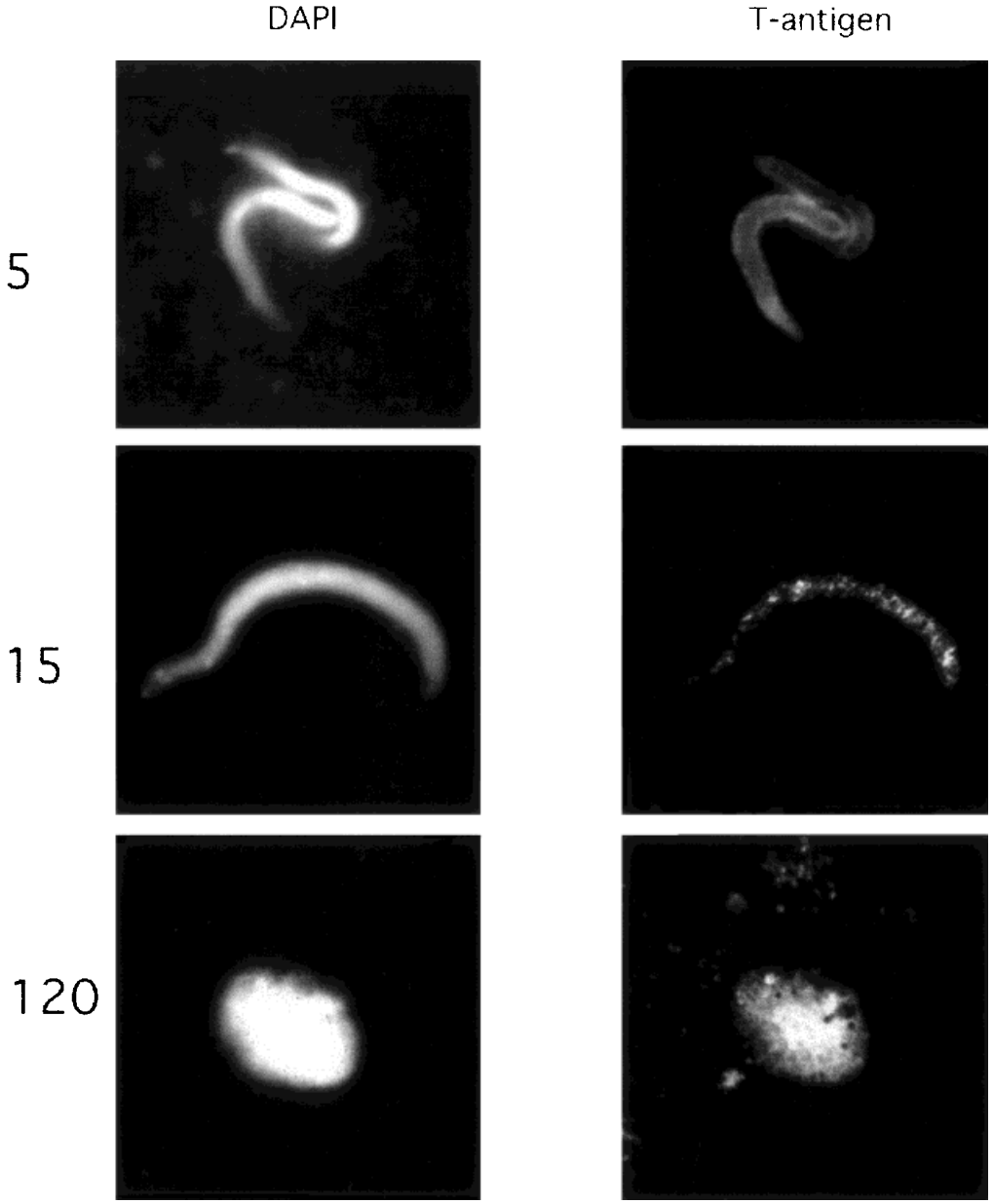

Fig. 1. T-antigen binding to nuclei in Xenopus egg extracts. Demembranated Xenopus sperm nuclei (2×10^5) were incubated in 100 µl Xenopus egg extracts containing 0.5 µg purified T-antigen, as described in Materials and Methods. Aliquots were taken at 0, 5, 15, 60, and 120 min, then the nuclei were fixed and stained with anti-T-antigen antibodies. DNA was visualized by DAPI staining.

[Tchang et al., 1993]. When sperm nuclei were incubated in Xenopus egg extracts supplemented with T-antigen, both T-antigen and p53 were localized within the nucleus, but no obvious colocalization of the two proteins at specific sites or structures of the nucleus was observed (Fig. 4). However, a partial colocalization would have been difficult to demonstrate by immunofluorescence, and we investigated for a possible association between T-antigen and the endogenous p53 at the biochemical level. Antibodies directed against T-antigen immunoprecipitated a substantial amount of endogenous p53 (Fig. 5A). In the reciprocal assay, antibodies directed against p53 also immunoprecipitated T-antigen. In contrast, no interaction with PCNA was detected (Fig. 5C), in agreement with the lack of colocalization by immunofluorescence analysis. We conclude that T-antigen interacts with the endogenous p53 store. This interaction mainly affects the soluble fraction of the protein since no clear colocalization of the proteins bound to chromatin was observed. In order to further analyze the interaction between T-antigen and p53, we have used centrifugation in sucrose gradients to isolate protein complexes involving T-antigen. Xenopus egg extract alone or containing T-antigen were incubated for 60 min at 23°C and then layered over a sucrose gradient and centrifuged. The fractions were collected, run on an SDS-polyacrylamide gel, transferred onto nitrocellulose filters, and probed with antibodies against T-antigen and p53. In the absence of extract, T-antigen sedimented essentially as a monomer (Fig. 6A). After incubation with the extract, T-antigen formed high molecular weight complexes with two main peaks sedimenting at 300 kDa and 650 kDa (Fig. 6B). The endogenous p53 sedimented as a dimer-like complex in the egg extract incubated in the absence of T-antigen (Fig. 6A), as observed in the early embryos (unpublished observations). When T-antigen was added to the extract, p53 shifted at the position in the gradient corresponding to the 300 kDa complex containing T-antigen. This observation is in agreement with the immunoprecipitation experiments showing that T-antigen interacts with the endogenous p53 present in the Xenopus egg.

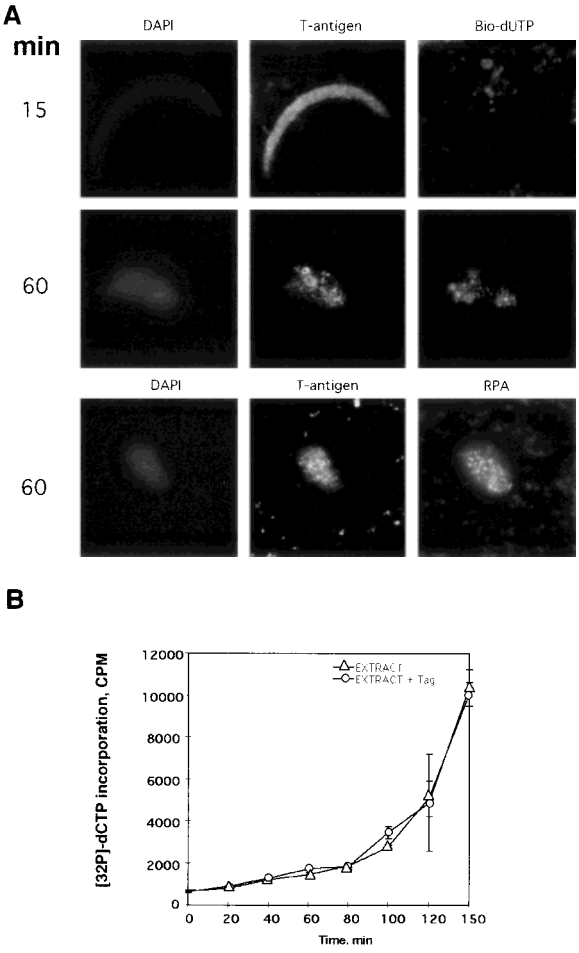

Fig. 2. **A:** T-antigen interaction with chromatin during DNA replication. Demembranated Xenopus sperm nuclei (2×10^5) were incubated in 100µl Xenopus egg extracts containing 0.5µg purified T-antigen in the presence of 7.5µM Biotin-dUTP, as described in Materials and Methods. The nuclei were fixed at 15 and 60 min and stained with anti-T-antigen, anti-RPA antibodies or streptavidin-Texas Red. DNA was visualized by DAPI staining. **B:** Efficiency of DNA replication in presence of T-antigen. Demembranated Xenopus sperm nuclei ($2x10^5$) were incubated in 100µl Xenopus egg extracts with or without 0.5µg purified T-antigen, in the presence of [^{32}P]-dCTP. 10µl-samples were taken at different times, treated as described in Materials and Methods and further analyzed by either electrophoresis followed by quantitation with a PhosphorImager (Molecular Dynamics, USA) or deposited onto Whatman GF/C glass filters, washed with TCA and counted in a liquid scintillation counter. The data shown represent three independent experiments.

Association of T-Antigen With the Nuclear Matrix

We next examined whether p53 and T-antigen associate in a subnuclear fraction non detectable by immunofluorescence studies of whole nuclei.

T-antigen is a component of the nuclear matrix in cells infected or transformed by SV40 [Staufenbiel and Deppert, 1983]. The association of T-antigen with chromatin incubated in Xenopus egg extract was analyzed at different stages of the cell cycle. Nuclei were isolated and

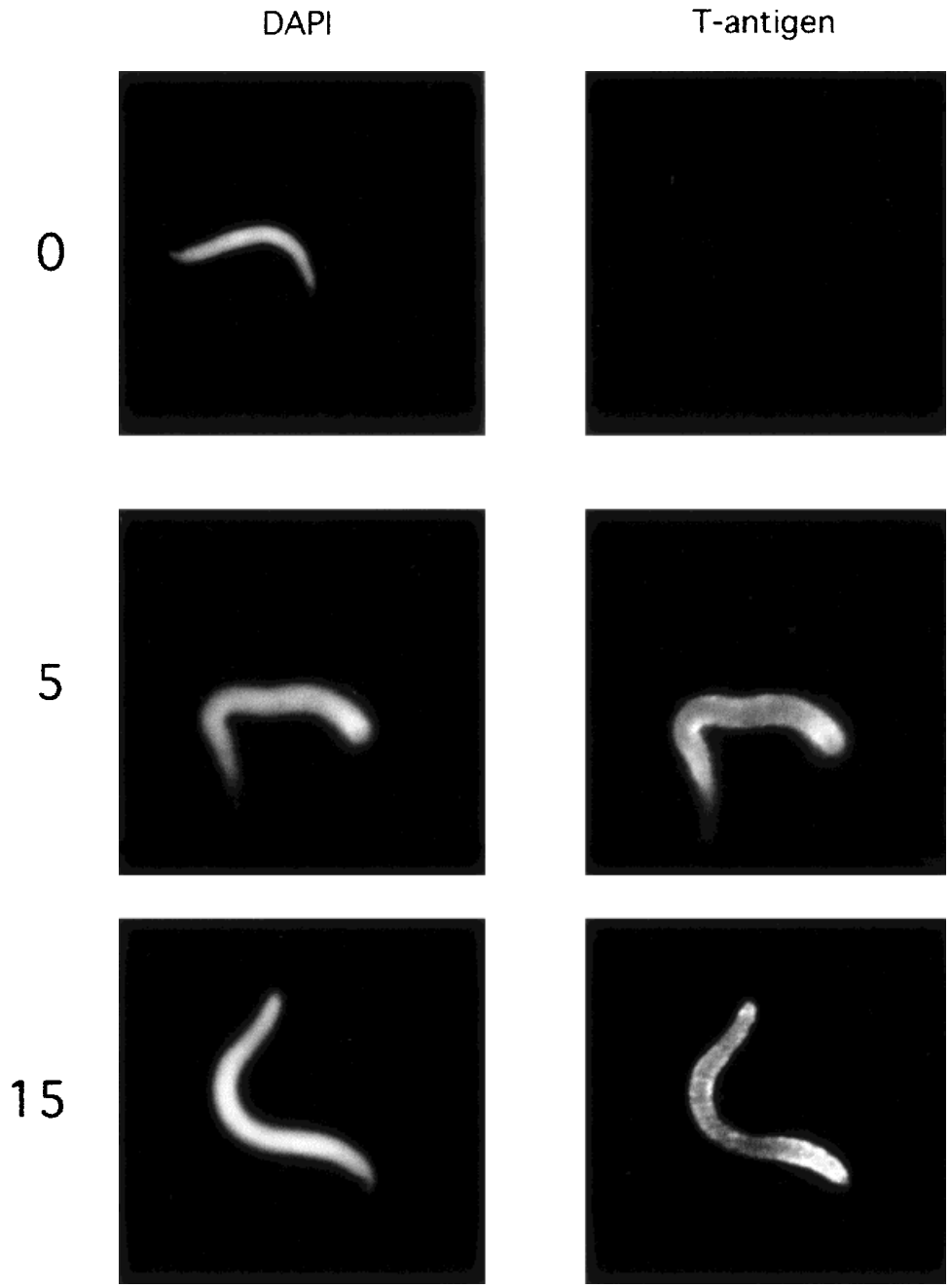

Fig. 3. T-antigen binding to chromatin in absence of nuclear membrane formation. Demembranated Xenopus sperm nuclei (2×10^5) were incubated in 100μl high speed Xenopus egg extract containing 0.5μg purified T-antigen. Aliquots were taken at 0, 5, and 15 min, then the nuclei were fixed and stained with anti-T-antigen antibodies. DNA was visualized by DAPI staining.

extracted with 1M NaCl, which removes histones from the chromatin, leading to its unfolding. We checked for the absence of residual histones in the nuclear matrix preparation (data not shown). The resulting loops of decondensed DNA remain attached to the nuclear matrix and form an external halo [Paulson and Laemmli, 1977]. Nuclei were then studied by immunofluorescence microscopy with antibodies directed against both T-antigen and p53. The data presented in Figure 7 indicates that, prior to initiation of DNA synthesis, T-antigen specifically interacted with the DNA loops extending from the nuclear matrix and was to-

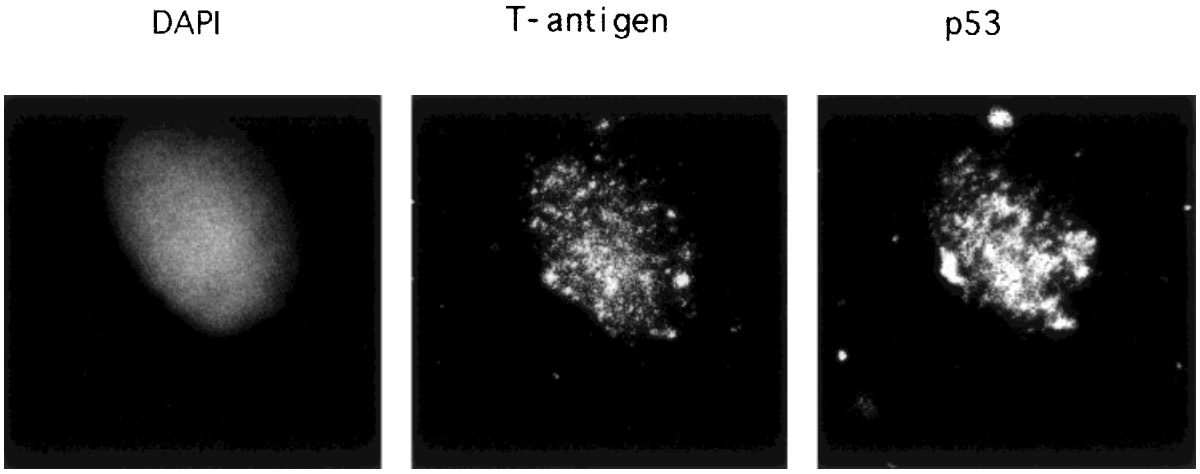

Fig. 4. Localization of T-antigen and p53 during the cell cycle. Demembranated Xenopus sperm nuclei (2×10^5) were incubated in 100µl Xenopus egg extract containing 0.5µg purified T-antigen and 7.5µM Biotin-dUTP. The nuclei were fixed at 60 min, stained with anti-T-antigen antibodies and anti-p53 antibodies. DNA was visualized by DAPI staining.

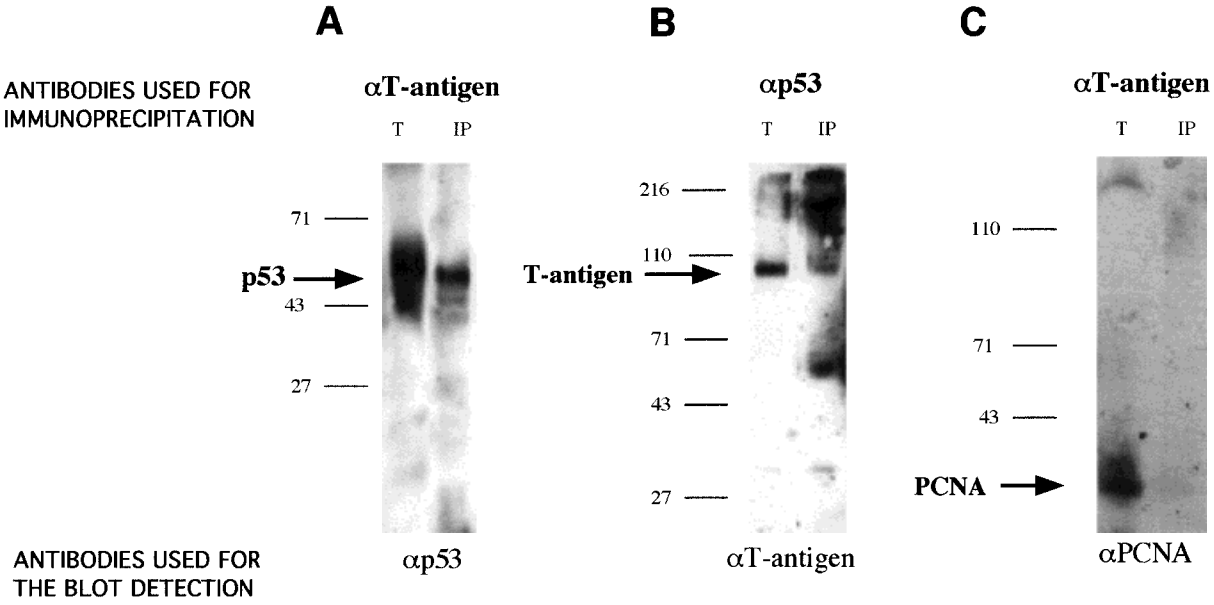

Fig. 5. Interaction of T-antigen and p53 detected by immunoprecipitation. 200µl Xenopus egg extract containing 0.5µg purified T-antigen was incubated for 60 min at 23°C and then immunoprecipitated using either anti-p53 or anti-T-antigen antibody. The immunoprecipitated material was washed and then separated by SDS-PAGE, transferred onto Hybond C+ membrane and visualized with either anti-p53 (**A**), anti-T-antigen (**B**), or anti-PCNA antibodies (**C**). T, total protein, IP, immunoprecipitated proteins.

tally excluded from the core nuclear matrix (Fig. 7, 15 min). No p53 staining was detected in the nuclear matrix at this stage. After 60 min, during S phase, we observed a transient association of T-antigen with the nuclear matrix. At this time p53 also became detectable in the nuclear matrix (Fig. 7, 60 min). A similar colocalization was detected during the G2 phase, when both T-antigen and p53 remained in the nuclear matrix (Fig. 7, 120 min).

To confirm this colocalization, we further analyzed the association of both T-antigen and p53 with the nuclear matrix by immunoblot detection of the proteins bound to the matrix at a time when active DNA synthesis occurs, after a 60 min incubation period. Both p53 and

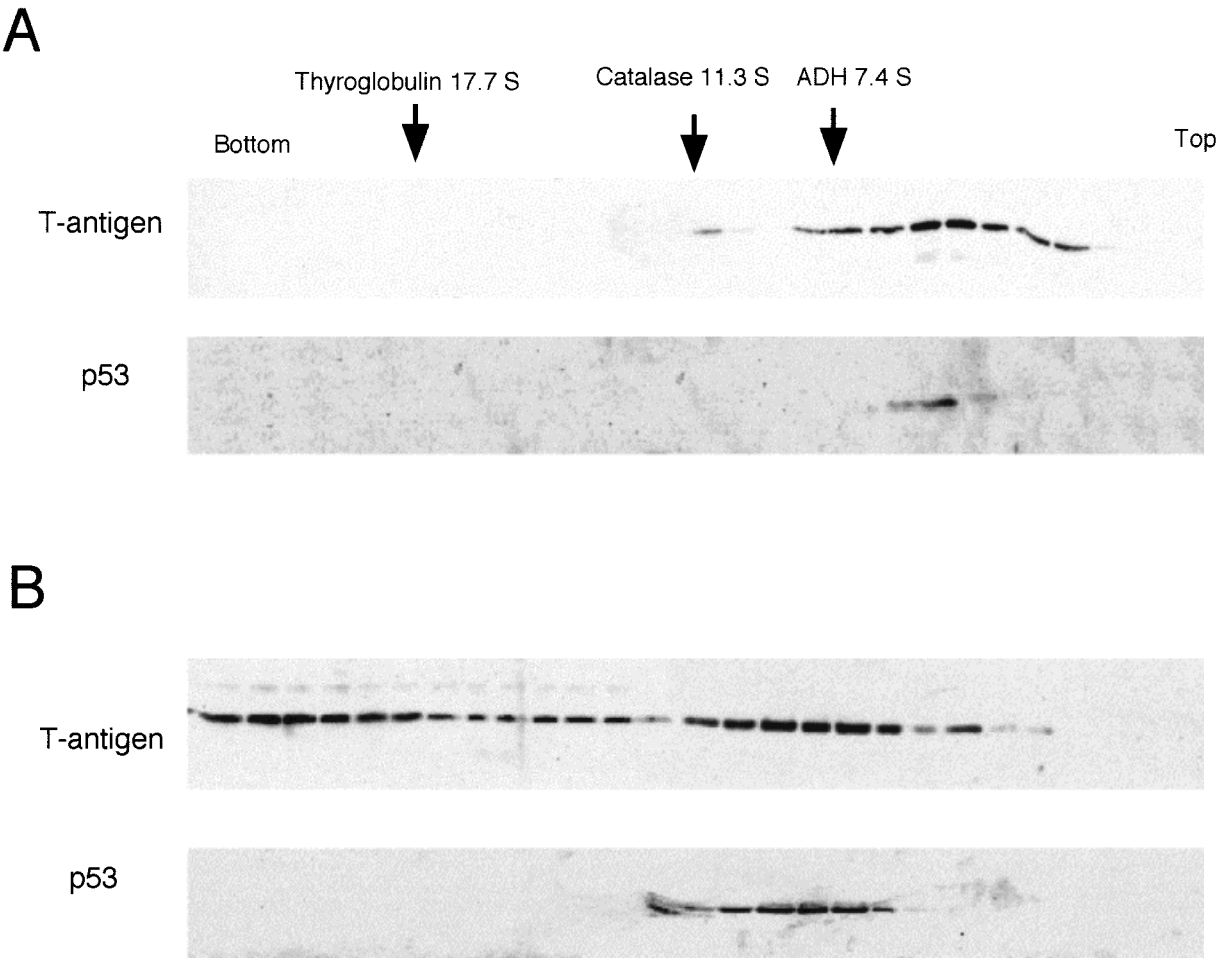

Fig. 6. T-antigen complexes containing T-antigen and p53 analyzed by sucrose gradient sedimentation. 200µl Xenopus egg extract containing 0.5µg purified T-antigen was incubated for 60 min at 23°C and then layered over a 15–30% sucrose gradient and centrifuged for 20h at 40,000 rpm and 4°C. The fractions were collected, run on an SDS-page, transferred onto nitrocellulose filters and probed with antibodies against T-antigen and p53.

T-antigen were detected associated with the DNa-seI-treated nuclear matrix (Fig. 8), although the association of T-antigen to the nuclear matrix was stronger. A substantial part of T-antigen (approximately 30%) remained bound to the matrix in 2M NaCl (Fig. 8B), whereas most of p53 was released from the matrix between 0.5 and 1M NaCl (Fig. 8A).

We conclude that both p53 and T-antigen associate with the nuclear matrix, and that a colocalization of the two proteins within the nuclear matrix occurs when S phase is initiated. These data may explain the difficulty to observe the colocalization in immunofluorescence studies on the whole nuclei (Fig. 4), and are consistent with the observed biochemical association of p53 with T-antigen detected in immunoprecipitation experiments as well as in sucrose gradients (Figs. 5 and 6).

DISCUSSION

T-antigen binds to the replication origin of SV40 and has two main functions, the recruitment of cellular replication machinery, and the unwinding of DNA strands at the replication origin. In non-permissive cells, T-antigen triggers the onset of S phase and cell transformation, possibly as a consequence of the repression of the antitumor activity of p53 [Moran, 1993].

Here, we used an in vitro system for eukaryotic chromosomal replication to investigate the subnuclear interaction of T-antigen with chromatin and replication centers. We have alsoanalyzed its association with p53, a protein

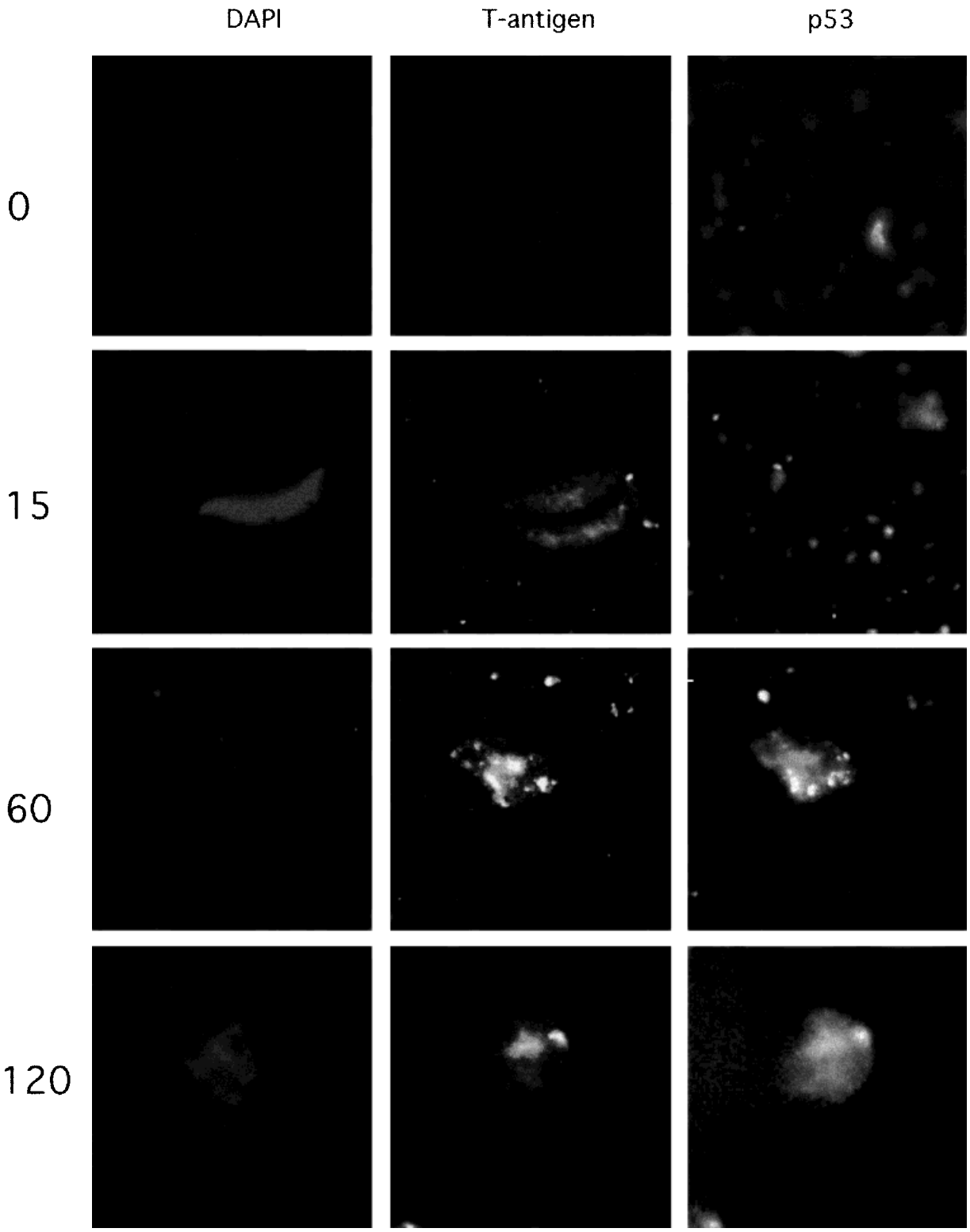

Fig. 7. Localization of T-antigen and p53 in the nuclear matrix during the cell cycle. Demembranated Xenopus sperm nuclei (2×10^5) were incubated in 100µl Xenopus egg extract containing 0.5µg purified T-antigen. Aliquots were taken at 0, 15, 60, and 120 min, the nuclei were spun onto glass cover slips and extracted with 1 M NaCl, fixed, and stained with T-antigen and p53 antibodies. DNA was visualized by DAPI staining.

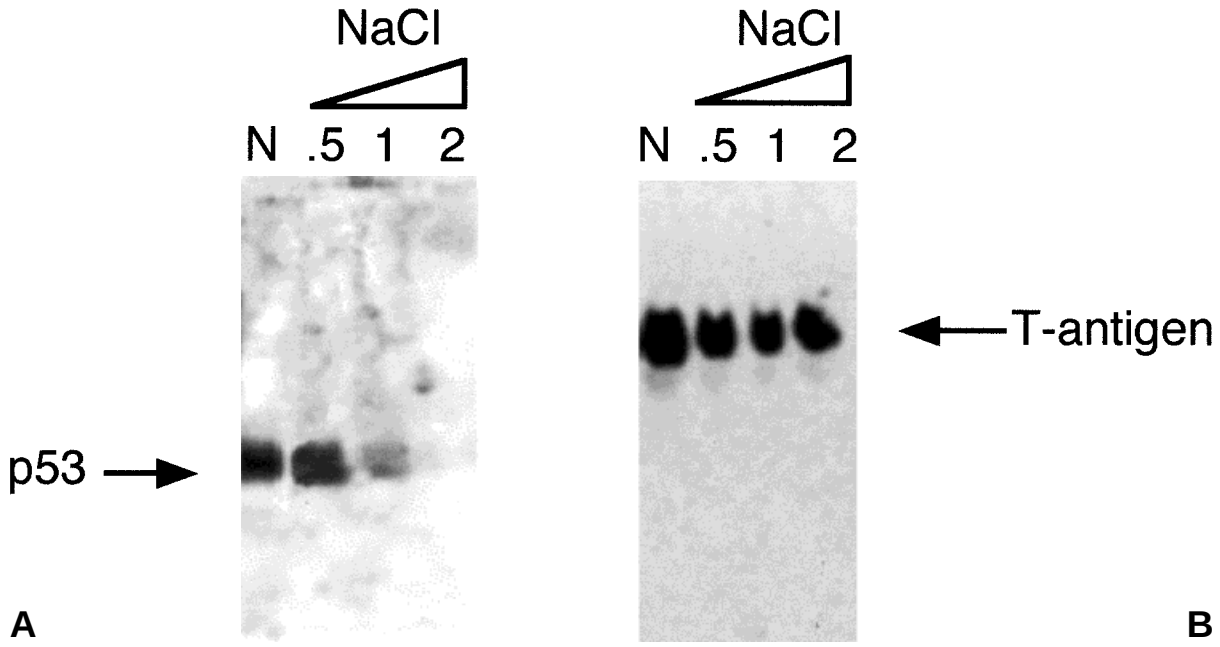

Fig. 8. Biochemical detection of the association of T-antigen and p53 with the nuclear matrix. Demembranated Xenopus sperm nuclei (10^6) were incubated in 100µl Xenopus egg extract containing 0.5µg purified T-antigen at 23°C for 60 min. The nuclear matrix was prepared by digestion with 100µg/ml DNase I for 30 min at 0°C followed by a stabilization step (incubation in the presence of 1 mM CuSO4) and extraction with a solution containing 20 mM Tris, 10 mM EDTA, and 0.5, 1, or 2 M NaCl for 30 min at 0°C. The nuclear matrices were then spun through the cushion of 0.8 M sucrose and analyzed by SDS-PAGE, transferred onto Hybond C+ membrane and hybridized with either anti-p53 (**A**) or anti-T-antigen antibodies (**B**). N, no extraction (control); 0.5, 1, and 2, extraction with 0.5, 1, and 2 M NaCl, respectively.

naturally present in large amounts in Xenopus egg extracts.

T-Antigen Binds to Chromatin Independently and Does Not Require a Nuclear Membrane for Its Nuclear Import

T-antigen contains a canonical nuclear localization signal [Fanning and Knippers, 1992], which permits its entry into the nucleus. Mutation studies demonstrated, however, that the presence of an NLS was not required forT-antigen-induced cell immortalization [Lanford et al., 1985]. We observed that SV40 T-antigen binds to chromatin before nuclear membrane formation occurs, and that this interaction is independent of the presence of nuclear membrane precursors. These data suggest that T-antigen may bind chromatin at the end of mitosis, when the nuclear envelope is still absent, and may explain why its nuclear localization signal is not essential for its transforming activity [Lanford and Butel, 1984; Lanford et al., 1985; Pannuti et al., 1987]. They also provide evidence that T-antigen can directly bind nuclear chromatin by mechanisms that are distinct from the active nuclear transport.

During DNA replication, we did not observe colocalization of T-antigen with the pre-replication and replication foci, nor did we detect any quantitative or kinetic effect of T-antigen on DNA replication. One possible explanation is the specificity of the functional interaction between DNA polymerase-alpha and T-antigen, responsible for the primate-specific replication of SV40 DNA [Dornreiter et al., 1990; Schneider et al., 1994; Stadlbauer et al., 1996]. However, microinjection of T-antigen induces host DNA synthesis in cell lines deriving from various species [Dickmanns et al., 1994], and expression of T-antigen in different mammalian cell lines that do not replicate SV40 DNA, promotes mitogenic cell transformation [Chen and Van Dyke, 1991]. Our observations indicate that T-antigen affects host DNA synthesis by a mechanism that is distinct from a direct interaction with the replication machinery.

Association of T-Antigen With the Nuclear Matrix and p53

Inhibition of T-antigen-dependent SV40 DNA replication in HeLa cell extracts containing ex-

ogenous T-antigen occurs by a mechanism that involves inhibition of the T-antigen helicase activity [Sturzbecher et al., 1988; Wang et al., 1989]. The addition of Wild-type human p53, however, has no effect on nuclear DNA replication in the Xenopus system [Cox et al., 1995 and our unpublished data]. These results are consistent with the large store of endogenous p53 already present during the early stages of embryonic development [Tchang et al., 1993] when cell division is extremely active.

We observed that both T-antigen and p53 are associated with the nuclease-treated and salt-extracted nuclei during S phase. A large part of the p53 store remains soluble in the Xenopus egg and the immunoprecipitation and sucrose gradient analysis reveals that this soluble fraction can also interact with T-antigen. However, such interaction of T-antigen with the endogenous pool of p53 does not promote a dominant negative effect on DNA replication. During viral infection or in T-antigen transformed cells, the positive effect of T-antigen on replication of both viral and host DNA has been explained by neutralization of p53. This regulation may not operate in the context of the early embryo, as DNA replication already occurs at an accelerated rate despite the presence of a large endogenous p53 pool. These results also reinforces the idea that p53 regulates the cell cycle by transcriptional regulation of specific target genes rather than directly interacting with the replication machinery. In the egg extract, all components required for the cell cycle are already accumulated and therefore the p53 control of the cell cycle is inefficient. In contrast, normal somatic cells divide more slowly and require transcriptional control and checkpoints which do not operate during the early development. In somatic cells, the targeting of endogenous p53 by T-antigen may then neutralize its antitumoral activity and explain the mitogenic role played by SV40 T-antigen in this process. The p53 control of the cell cycle may therefore be subjected to substantial variations, according to the cell lineage and genetic background.

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