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Transcription Factor Runx1 Recruits the Polyomavirus Replication Origin to Replication Factories

Yota Murakami,[1]* Ling-Feng Chen,[1] Noriyuki Sanechika,[1] Hidetsugu Kohzaki,[1,2] and Yoshiaki Ito[1]

[1]Department of Viral Oncology, Institute for Virus Research, Kyoto University, Sakyo-ku, Kyoto 606-8507, Japan

[2]Venture Laboratory, Kyoto Institute of Technology, Matsugasaki, Sakyo-ku, Kyoto 606-8585, Japan

Abstract Eukaryotic DNA replication takes place in the replication factories, where replication proteins are properly assembled to form replication forks. Thus, recruitment of DNA replication origins to the replication factories must be the key step for the regulation of DNA replication. The transcription factor Runx1 associates with the nuclear matrix, the putative substructure of DNA replication factories. An earlier report from our laboratory showed that Runx1 activates polyomavirus DNA replication, and that this requires its nuclear matrix-binding activity. Here, we show that Runx1 activates polyomavirus DNA replication by stimulating the binding of the viral-encoded replication initiator/helicase, large T antigen, to its replication origin. We found that newly replicated polyomavirus DNA is associated with the nuclear matrix and that large T antigen is targeted to replication factories, suggesting that polyomavirus is replicated in replication factories on the nuclear matrix. Although Runx1 did not co-localize with large T antigen-containing foci by itself, it co-localized with large T antigen-containing replication factories during Runx1-dependent polyomavirus DNA replication. These observations together suggest that Runx1 recruits the polyomavirus replication origin to the replication factory on the nuclear matrix, and that this requires the nuclear matrix-binding activity of Runx1. J. Cell. Biochem. 100: 1313–1323, 2007.

Key words: DNA replication; nuclear matrix; polyomavirus; replication factory; transcription factor

It has been recognized recently that the structure of the nucleus plays important roles in the regulation of nuclear functions. The nuclear matrix, which is also referred to as the nucleoskeleton or nuclear scaffold depending on the isolation procedure used, has been described as a network of fibrous nucleoproteins present throughout the nucleus [Berezney et al., 1995; Jackson, 2003]. Compelling evidence suggests that the nuclear matrix is needed as a framework to organize DNA and RNA synthesis in the nucleus [Cook, 1999; Jackson, 2003; Stein et al., 2003; Anachkova et al., 2005]. These studies have shown that discrete "factories" for each process, in which the necessary proteins are organized in a functionally appropriate manner, are formed on the nuclear matrix [Cook, 1999]. The DNA replication factories consist of DNA polymerases, PCNA, and other replication proteins, which eventually form replication forks [Hozak et al., 1993; Cardoso and Leonhardt, 1998]. This indicates that replication origins, where replication forks form initially, have to associate with the replication factory at some point before DNA replication is initiated [Cook, 1999]. However, the mechanism underlying the association between replication origins and the replication factory remains unclear.

Transcription factors regulate the formation of transcriptional complexes on specific promoters on the chromatin. To achieve this function, different transcription factors have distinct functions, including the recruitment of component(s) of the transcriptional complex and

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Ling-Feng Chen's present address is Department of Biochemistry, University of Illinois, 600 S Mathews Avenue, Urbana, IL 61801.

Yoshiaki Ito's present address is Institute of Molecular and Cell Biology, 61 Biopolis Drive, Proteos 138673, Singapore.

*Correspondence to: Yota Murakami, Institute for Virus Research, Kyoto University, Sakyo-ku, Kyoto 606-8507, Japan. E-mail: yota@virus.kyoto-u.ac.jp

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alteration of chromatin structure. In addition, a recent study revealed that some transcription factors localize to the nuclear matrix and that this localization is important for their functions. These factors include the Runx family transcription factors, which have been shown to occupy discrete foci on the nuclear matrix [Zeng et al., 1997; Chen et al., 1998], the majority of which coincide with transcription sites [Zeng et al., 1998; Harrington et al., 2002]. Runx proteins also contain a nuclear matrix-targeting domain, and it has been shown that this domain is important for their transcriptional activation function [Zeng et al., 1997].

Accumulating evidence indicates that transcription factors also regulate DNA replication [Murakami and Ito, 1999; Kohzaki and Murakami, 2005]. Polyomavirus (Py), which replicates in a transcriptional enhancer-dependent manner [de Villiers et al., 1984], is a good model system for investigating the role of transcription factors in DNA replication. Runx1 was originally isolated as a protein binding to the "core" sequence of Py enhancer [Kamachi et al., 1990], which is important for Py DNA replication stimulatin [Veldman et al., 1985]. We have shown that Runx1 activates Py DNA replication [Chen et al., 1998]. Interestingly, the replication activation domain of Runx1 (RAD) has nuclear matrix-binding activity and this activity is required for it to stimulate Py DNA replication [Chen et al., 1998]. We also showed previously that the transcription factor c-Jun activates Py DNA replication by directly recruiting of the viral initiator/helicase, large T antigen, to the replication origin [Murakami et al., 1991; Ito et al., 1996]. However, we have shown that Runx1 does not interact with large T antigen [Chen et al., 1998]. Thus, the molecular mechanism by which Runx1 activates DNA replication and the role the nuclear matrix binding of Runx1 plays in this mechanism remains unclear.

Here we show that RAD stimulates the binding of large T antigen to the Py origin in vivo and that this stimulation requires the nuclear matrix-binding activity of RAD. In addition, we found that while large T antigen is targeted to the cellular replication factory, Runx1 itself co-localizes with the large T antigen-containing replication foci only when Py DNA replication is taking place. These results are consistent with a model in which Runx1 recruits the Py replication origin to the replication factory.

MATERIALS AND METHODS

Cells, Antibodies, and Plasmids

Mouse fibroblast NIH3T3 cells and COP5 cells [Tyndall et al., 1981] were grown in DMEM supplemented with 10% calf serum. Transfection of plasmid DNA was performed with Fugene 6 (Roche) according to the manufacturer's instructions. Anti-Py large T antigen mouse monoclonal antibody (Ab4, CALBIOCHEM) and anti-HA mouse monoclonal antibody (12CA5, Roche) were used for the ChIP assays. For immunofluorescence analysis, we used anti-Py large T antigen hamster polyclonal antibody (Y. Ito, unpublished), anti-PCNA mouse monoclonal antibody (PC10, DAKO), anti-HA rabbit polyclonal antibody (CAPEL Research Reagents), and secondary antibodies coupled with Cy3, FITC, and Cy5 (Jackson Immuno Research).

Plasmids

Expression plasmids for Py large T antigen, HA-tagged Runx1, GAL4-RAD and GAL4-VP16, and the reporter plasmids pPyOIcat, pPyBPPOIcat, pPy$(AE)_4$OIcat, and pPyG5OIcat have been described previously [Murakami et al., 1990; Chen et al., 1998]. pHSG398 (Takara) was previously described [Takeshita et al., 1987].

Chromatin Immunoprecipitation Assay

The assay was performed as described previously [Orlando and Paro, 1993]. Briefly, 24 h after transfection, about 5×10^7 sub-confluent cells grown in 200-mm diameter dishes were fixed with 1% formaldehyde for 20 min at 37°C. The cells were then collected and 1 ml of extract was prepared as described by Orlando and Paro [1993]. We omitted a CsCl isopycnic centrifugation step from the original procedure [Orlando and Paro, 1993]. Immunoprecipitation was performed using 0.5 ml of extract (corresponding to about 2.5×10^7 cells), 2 μg of anti-Py large T antigen monoclonal antibody or anti-HA monoclonal antibody, and Dynabeads M280 conjugated to anti-mouse IgG antibody (Dynal). After removing the cross-links by overnight incubation at 65°C and proteinase K digestion,

the DNA in the precipitates was purified by phenol-chloroform extraction and ethanol precipitation, and then suspended in 20 μl of distilled water. To prepare the DNA from whole cell extract, the DNA from 100 μl of extract was purified after removing the cross-links and suspended in 100 μl of distilled water. One microliter each of the immunoprecipitated DNA or the whole cell extract DNA was used for PCR. The primers used in the PCR were oriE (5′-GAATTCTGCCGCCGGGCCTCTTGCGGG-3′) and oriL (5′-GAATTCGCCTCTCTTCTTTTTC-TCCAGAG-3′). The amplified DNA was separated on 2% agarose gels, stained with Et-Br, and quantified on a Fluor-S MultiIImager (Bio-Rad).

Analysis of Newly Synthesized DNA

NIH3T3 cells (2×10^7) were grown in DMEM supplemented with 10% calf serum in 20-mm diameter dishes before transfection with the indicated plasmids by using Fugene 6 (Roche). Twenty-four hours after transfection, the cells were washed twice with ice-cold PBS and extracted with 2 ml of ice-cold CSK buffer [Orlando and Paro, 1993] containing 0.1% Triton X. The extract was used as the soluble fraction. The pellet was suspended in digestion buffer (100 mM NaCl, 10 mM Tris-HCl pH 8.0, 2.5 mM EDTA, 0.25% SDS). The suspension and the soluble fraction were adjusted to 0.25% SDS and treated with 0.1 mg/ml proteinase K. The DNA was then purified by phenol-chloroform extraction and RNaseA treatment. After ethanol precipitation, the DNA was suspended in 100 μl of TE buffer (10 mM Tris-HCl pH 8.0, 1 mM EDTA) and 20 μl was digested with *Hind*III with or without *Dpn*I. The digested DNA was blotted onto Hybond N (GE Health Care) after 0.7% agarose gel electrophoresis. The plasmid DNA was detected by hybridization with a fragment-containing part of pPyOIcat and pHSG398 as described before [Murakami et al., 1990].

Immunofluorescence of Nuclear Matrix-Binding Proteins

The association of proteins with the nuclear matrix was determined by immunofluorescence microscopy with Micro Radiance confocal microscope (Bio Rad) as described previously [Fey et al., 1984].

RESULTS

The RAD of Runx1 Is Needed for its Stimulation of Large T Antigen Binding to the Core Origin

Py enhancer stimulates Py DNA replication. Since binding of initiator protein to DNA replication origin is the key step for the regulation of the initiation of DNA replication, we analyzed the effect of the Py enhancer on large T antigen binding to the core origin in vivo, using the chromatin immunoprecipitation (ChIP) assay. COP5 cells expressing Py large T antigen [Tyndall et al., 1981] were transfected with a Py origin-containing plasmid (Fig. 1A), and the control plasmid pUC19 and the binding of large T antigen to the core origin was examined (Fig. 1B). Large T antigen bound to the core origin of pPyBPPOIcat (lane 3) having the native enhancer sequence (Fig. 1A) [Murakami et al., 1990], but not to the core origin of pPyG5OIcat (lane 6) harboring five copies of the binding site for the yeast transcription factor GAL4 (Fig. 1A). This indicates that a transcriptional enhancer is required for the binding of large T antigen to the core origin. Previously, we showed that one of the Py enhancer-binding proteins, Runx1, stimulates Py DNA replication and that RAD, replication activation domain of Runx1, fused to the GAL4 DNA-binding domain (GAL4-RAD) stimulates Py DNA replication when it becomes tethered close to the core origin [Chen et al., 1998]. Therefore, we examined whether GAL4-RAD stimulates the T antigen binding like Py enhancer. For this, we used plasmids containing the core origin with or without five copies of the GAL4-binding site (pPyG5OIcat and pPyOIcat, respectively, Fig. 1A). NIH3T3 cells were co-transfected with both plasmids along with the large T antigen expression plasmid together with the GAL4-RAD-expressing plasmid. The binding of large T antigen to the core origin was then analyzed by the chromatin immunoprecipitation (ChIP) assay (Fig. 1C). In the presence of the GAL4-RAD fusion protein, the core origin of pPyG5OIcat, but not that of pPyOIcat (lane 3) was observed in the immunoprecipitated fraction. This indicates that large T antigen preferentially binds to the GAL4 site-bearing core origin in the presence of GAL4-RAD. In the absence of GAL4-RAD, neither plasmid bound to large T antigen (lane 9). RAD is required for T antigen binding because expression of the GAL4 DNA-binding domain

alone did not promote the binding event (lane 6). These results clearly indicate that GAL4-RAD stimulates the binding of large T antigen to the core origin in a RAD-dependent manner.

Since RAD has nuclear matrix localization activity, GAL4-RAD localizes exclusively to the nuclear matrix [Chen et al., 1998]. AML1-ETO is a chimeric protein generated by the t(8;21) chromosome translocation found in acute myeloid leukemia. In AML1-ETO, the DNA-binding domain of AML (the human homolog of Runx1) is fused to the nuclear protein ETO [Erickson et al., 1992; Miyoshi et al., 1993]. Interestingly, AML1-ETO not only blocks the GAL4-RAD-dependent replication of Py DNA, it also inhibits the localization of GAL4-RAD to the nuclear matrix though the precise mechanism remains unclear [Chen et al., 1998]. Since the nuclear matrix localization of GAL4-RAD was well correlated with the GAL4-RAD-dependent replication activity in the inhibition experiments with AML1-ETO, we concluded that the nuclear matrix-binding activity of RAD is necessary for its ability to stimulate Py DNA replication [Chen et al., 1998]. Thus, we next asked if AML1-ETO also inhibits the RAD-dependent binding of large T to the core origin. We expressed AML1-ETO with GAL4-RAD and examined the binding of T antigen to the core origin by the ChIP assay (Fig. 1D). Co-expression of AML1-ETO inhibited the binding of large T antigen in a dose-dependent manner (lanes 6, 8, and 10). This inhibition by AML1-ETO was specific to RAD because AML1-ETO did not affect the enhanced binding of large T antigen mediated by GAL4-VP16 (lanes 14, 15, and 16), which also localizes to nuclear matrix and activates Py DNA replication but the both

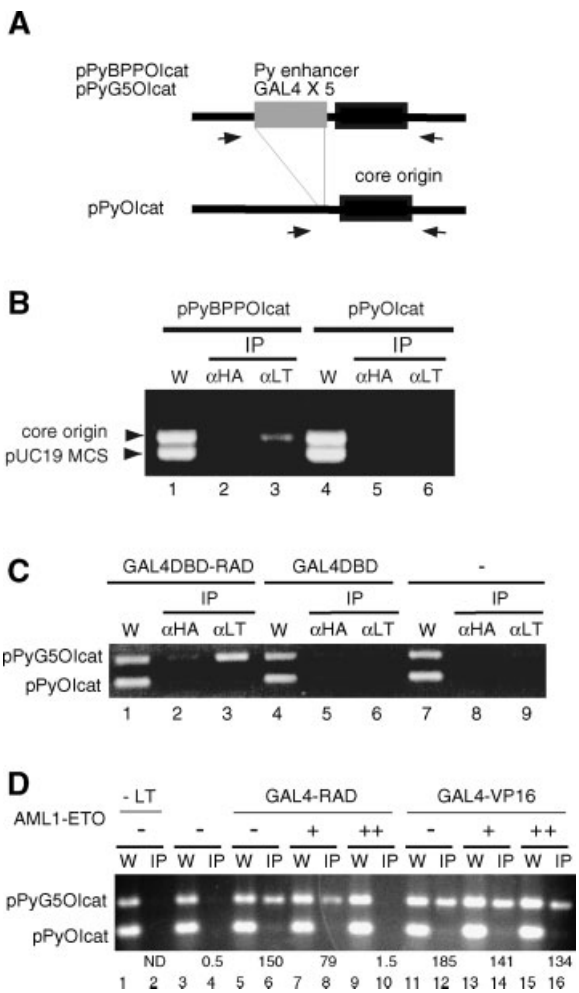

Fig. 1. The RAD of Runx1 stimulates large T antigen binding to the core origin. **A**: Schematic depiction of the reporter plasmids used in the assay. All contain the Py core origin (black box). In addition, pPyBPPOIcat and pPyG5OIcat contains the Py enhancer sequence and five copies of the GAL4-binding site (gray box) next to the core origin, respectively. The arrowheads indicate the primers used in PCR. **B**: A transcriptional enhancer is required for the binding of large T antigen to the core origin. Enhancer-containing plasmid (0.25 μg) pPyBPPOIcat or pPyOIcat, which lacks the enhancer, was transfected into COP5 cells along with 0.125 μg each of a plasmid expressing the Py large T antigen and the control plasmid (pUC19). The binding of large T antigen to the core origin was examined by ChIP assays (Materials and Methods) employing anti-Py large T antigen monoclonal antibody (αLT) or anti-HA monoclonal antibody (αHA) as a control. The primer set amplifies the core origin region of each plasmid as well as the multi-cloning sites (MCS) of pUC19, which served as the control plasmid. The positions of the PCR products representing the core origin and MCS of pUC19 in agarose electrophresis are indicated. W, products of PCR with DNA isolated from the whole cell extract. **C**: Requirement of the RAD of Runx1 for the binding of large T antigen to the replication origin. NIH3T3 cells were co-transfected with 1 μg of large T antigen expression plasmid and 0.25 μg each of pPyOIcat and pPyG5OIcat with or without 1.5 μg of the plasmid expressing GAL4-RAD (GAL4DBD-RAD) or GAL4 DNA binding domain (GAL4DBD). The ChIP assay was performed using anti-Py large T antigen antibody or, as a control, anti-HA antibody (αHA) to immunoprecipitate the cross-linked protein-bound DNAs. W, DNA from whole cell extract; IP, DNA from immunoprecipitates. **D**: AML1-ETO inhibits the RAD-dependent binding of T antigen to the origin. pPyG5OIcat, pPyOIcat, and the large T antigen expression plasmid were co-transfected into NIH3T3 cells together with the indicated expression plasmids as described in (B) and the binding of large T antigen was examined by the ChIP assay. + and ++ indicate that 1.5 and 3.0 μg of the expression plasmid for AML1-ETO were transfected, respectively. Note that the large T antigen expression plasmid was omitted from the transfection in **lanes 1** and **2** (-LT). The ratio of the pPyG5Oicat signal to that of pPyOIcat in the immunoprecipitate (IP) normalized with the ratio in the whole cell extract (W) is indicated beneath each IP lane.

activity were not affected by AML1-ETO [Chen et al., 1998]. From these results, we concluded that the nuclear matrix-binding activity of RAD is necessary for the RAD-mediated stimulation of large T antigen binding to the core origin.

Py Replicates at the Replication Factory on the Nuclear Matrix

Newly synthesized chromosomal DNA has been shown to preferentially associate with the nuclear matrix [Ortega and DePamphilis, 1998], which supports the notion that replication factories exist on the nuclear matrix. We examined the attachment of newly synthesized Py DNA to the nuclear matrix by cell fractionation (Fig. 2A). We co-transfected NIH3T3 cells with the Py T antigen and/or Runx1 expression plasmids along with the reporter plasmid pPy(AE)$_4$OIcat, which contains the core origin adjacent to six copies of Runx1-binding site. Twenty-four hours after transfection, whole cell extract was prepared and fractionated into Triton-soluble and -insoluble fractions (see Materials and Methods). The Triton-insoluble fraction is rich in nuclear matrix. The DNA from each fraction was extracted and digested with *Dpn*I, which cuts GATC sequences only when the adenine residues of both strands are methylated and *Hind*III, which cuts the reporter and control plasmid at a unique site. Unlike *E. coli*, mammalian cells do not have *dam* methylase, which methylates the A of the GATC sequence. Therefore, when the plasmid DNA isolated from *E. coli* is replicated in NIH3T3 cells, it becomes resistant to *Dpn*I digestion. The amount of the plasmid in each fraction was examined by Southern blotting (Fig. 2). Without *Dpn*I digestion (Fig. 1, upper panel), both the reporter plasmid pPy(AE)$_4$OIcat and the control plasmid pHSG398 were recovered from the Triton-soluble and -insoluble fractions, and the distribution was not affected by the expression of large T antigen and/or Runx1 (lanes 2–5 and 7-4). In contrast, with *Dpn*I digestion, the reporter plasmid could not be detected in the Triton-soluble fraction regardless of the expression of large T antigen and Runx1 (Fig. 2, lower panel, lanes 1–5), indicating that it had not been replicated. In the nuclear matrix-containing insoluble fraction, however, the reporter plasmid was resistant to *Dpn*I digestion, but only when the large T antigen- and Runx1-expressing plasmids were transfected together (lane 10). When the large T antigen-expressing plasmid was co-transfected on its own, only very small amounts of *Dpn*I-resistant reporter

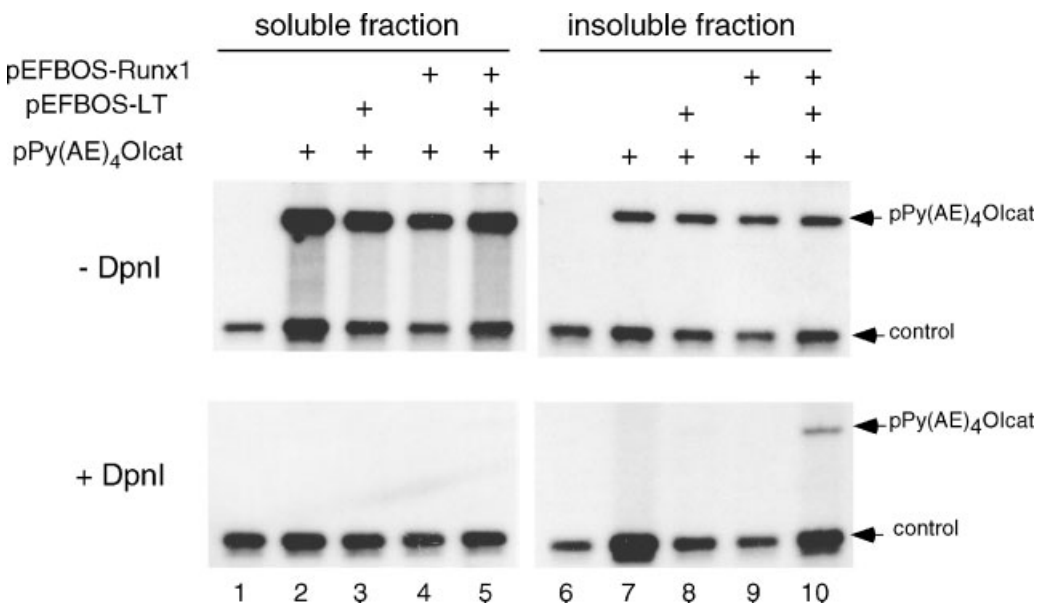

Fig. 2. Newly synthesized Py DNA localizes in the nuclear matrix-rich insoluble fraction. NIH3T3 cells were co-transfected with 5 μg of reporter plasmid pPy(AE)$_4$OIcat and 5 μg of control pHSG398 together with the indicated expression plasmids (3 μg each). Twenty-four hours after transfection, the cells were harvested and fractionated into Triton-soluble (**lanes 1–5**) and -insoluble fractions (**lanes 6–10**) as described in Materials and Methods. The DNA extracted from each fraction was digested with *Hind*III, which cuts the reporter and control plasmids once (upper panel) or with *Hind*III and *Dpn*I (lower panel). The *Dpn*I-resistant bands of the reporter plasmids represent the newly replicated molecules. The control plasmid pHSG398 is resistant to *Dpn*I because the DNA was prepared from *dam*$^-$ *E. coli*.

plasmid were detected (Fig. 2 lower panel, lane 7). This indicates that exogenously expressed Runx1 stimulates Py DNA replication. When large T antigen was absent, we could not detect any *Dpn*I-resistant plasmid, confirming that *Dpn*I-resistant DNA truly represents replicated Py DNA (compare lanes 9 and 10). These results indicate that replicated Py DNA preferentially attaches to the nuclear matrix. This is consistent with the idea that Py replicates at the replication factories like cellular DNA.

If Py replication takes place at the replication factories, large T antigen, an essential viral protein for the initiation and elongation of Py DNA replicaion, should localize to replication factories. To test this, we compared the localization of large T antigen with that of PCNA, an essential DNA replication factor that clearly localizes to replication factories [Leonhardt et al., 2000]. We transfected NIH3T3 cells with the large T antigen expression plasmid and examined the localization of exogenous large T antigen and endogenous PCNA on the nuclear matrix by immunofluorescence using a specific antibody against each protein and confocal microscopy (Fig. 3). PCNA formed clear foci in the 20%–30% of cells, which represent replication factories in S phase cells (Fig. 3A–C). T antigen also formed clear foci on the nuclear matrix irrespective of the presence or absence of PCNA foci (Fig. 3A–D). Interestingly, T antigen foci co-localized with PCNA foci in PCNA-positive cells (Fig. 3A–C). Since Py DNA was not co-transfected, no Py DNA replication took place in this experiments. Thus, this result indicates that large T antigen has an intrinsic ability to target the cellular replication factory.

Runx Co-Localizes With PCNA and Large T Antigen Foci Only When Runx1-Dependent Py DNA Replication Can Occur

We then compared the localization of large T antigen with that of Runx1 on the nuclear matrix. We expressed HA-tagged Runx1 and large T antigen simultaneously in NIH3T3 cells and examined the localization of both proteins on the nuclear matrix as shown in Figure 4A. As previously reported, Runx1 formed foci on the nuclear matrix; however, the foci did not overlap with those of large T antigen (Fig. 4A, no plasmid). Co-transfection of pPyOIcat, which has the core origin but lacks the Runx1-binding site and the ability to replicate efficiently [Murakami et al., 1990], had no effect on this unmatched localization of the two proteins (Fig. 4A, pPyOIcat). However, when the replication competent plasmid pPy$(AE)_4$OIcat (Fig. 2, lane 10), which has the core origin and Runx1-binding sites (Fig. 1A), was co-transfected into the cells, we observed clear co-localization of the T antigen foci and Runx1 foci (Fig. 4A, pPy$(AE)_4$OIcat). Thus, the replication-competent plasmid pPy$(AE)_4$OIcat mediates co-localization of large T antigen and Runx1. This suggested that Runx1 only becomes targeted to large T antigen-containing replication factories when Runx1-dependent Py DNA replication is taking place. To confirm this, we compared the localization of Runx1 and large T antigen with PCNA in the presence of pPy$(AE)_4$OIcat (Fig. 4B). We found that the foci of all three proteins clearly overlapped, which confirms that Runx1 is targeted to large T antigen-containing replication factories when Runx1-dependent Py DNA replication is occurring.

DISCUSSION

On the basis of the results presented here, we propose a model for how Runx1 stimulates Py DNA replication (Fig. 5). First, large T antigen is targeted to the replication factory on the nuclear matrix (Fig. 3). Second, in the absence of Py replication, Runx1 forms distinct foci on the nuclear matrix that do not coincide with replication factory foci (Fig. 4A); these Runx1 foci may instead represent transcription sites [Zeng et al., 1998; Harrington et al., 2002]. Third, large T antigen alone binds only poorly to the core origin without the assistance of transcription factors in vivo (Fig. 1). Thus, we speculate that Runx1 acts by binding to Py plasmid DNA harboring Runx1-binding sites and that this anchors the DNA to the nuclear matrix through the nuclear matrix-binding activity of RAD. Detailed analysis of replication and transcription foci in the nuclei has indicated that both sites are juxtaposed and probably co-organized [Hassan et al., 1994; Wei et al., 1998; Schwaiger and Schubeler, 2006]. Thus, we speculate that the Runx1-driven localization of the Py replication origin to the nuclear matrix helps the origin to encounter large T antigen in the replication factories (Fig. 5). In addition, Runx1 may even directly facilitate large T antigen binding to the replication factories. In line with this, we previously

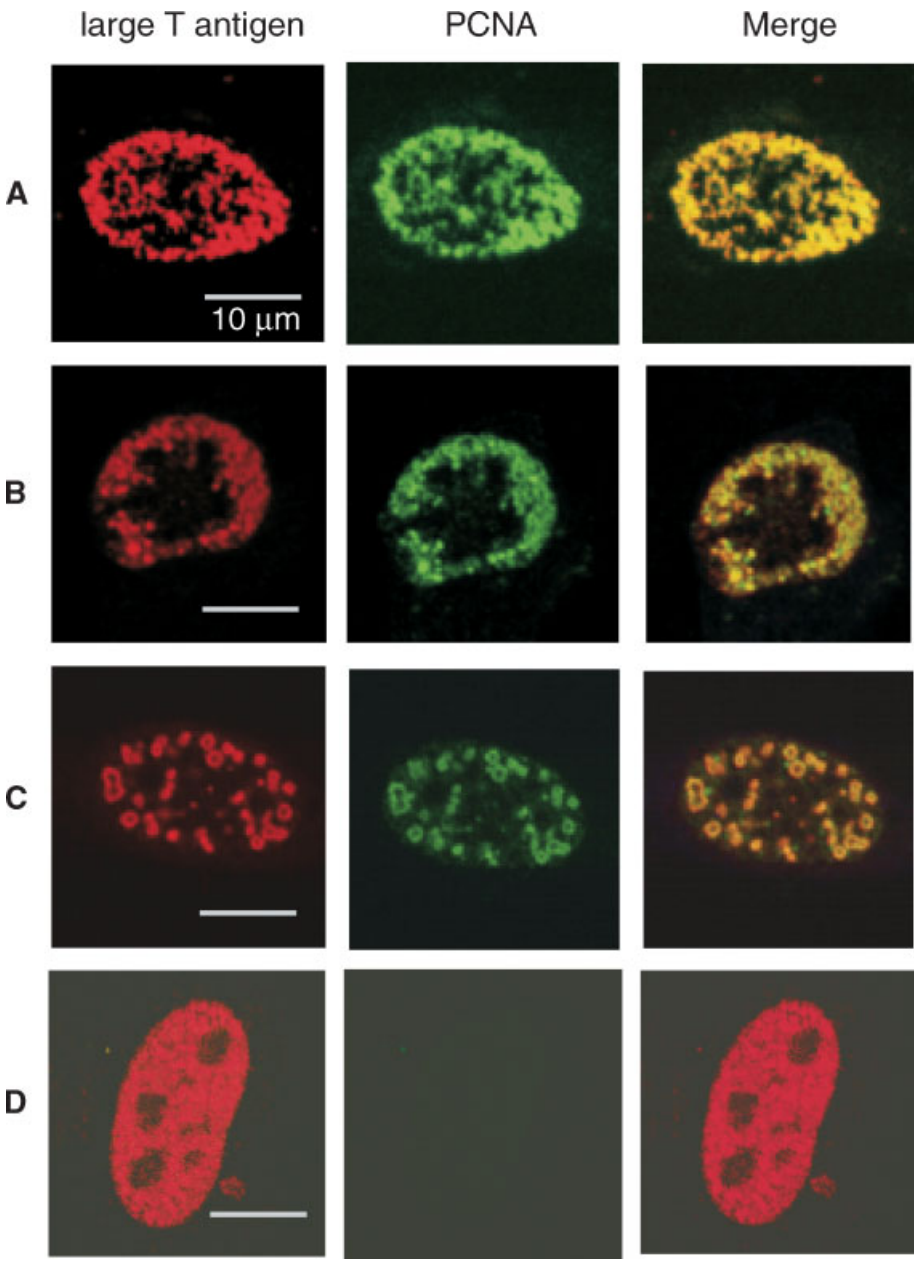

Fig. 3. Co-localization of Py large T antigen with PCNA in replication factories. NIH3T3 cells were transfected with the plasmid expressing Py large T antigen (1.5 μg), and the localizations of exogenous large T antigen (Red, Cy3) and PCNA (Green, FITC) on the nuclear matrix were visualized by immunofluorescence. Typical staining patterns are shown. Small PCNA foci were either distributed throughout the nucleoplasm with the exception of the nucleoli (**A**) or concentrated in the nuclear periphery (**B**); these distributions represent the early S-phase and mid S-phase, respectively. In late S-phase, the PCNA foci decreased in number but increased in size, often taking on characteristic ring and horseshoe-like structures (**C**). At all stages of the S phase, the large T antigen foci co-localized with the PCNA foci (merged panels). T antigen also formed foci in the nuclei that did not show PCNA foci (**D**).

indicated that nuclear matrix targeting alone is not enough for the Py DNA replication. We isolated the mutation in RAD that disrupted the DNA replication activity but did not affect the nuclear matrix binding. The wild type and mutated RAD competed with Runx1 for both nuclear matrix binding and replication activity in vivo, but the competition by the wild-type RAD for the replication activity was severer than that by the mutated RAD. This strongly suggested that RAD interacts with replication factors to stimulate Py DNA replication and the

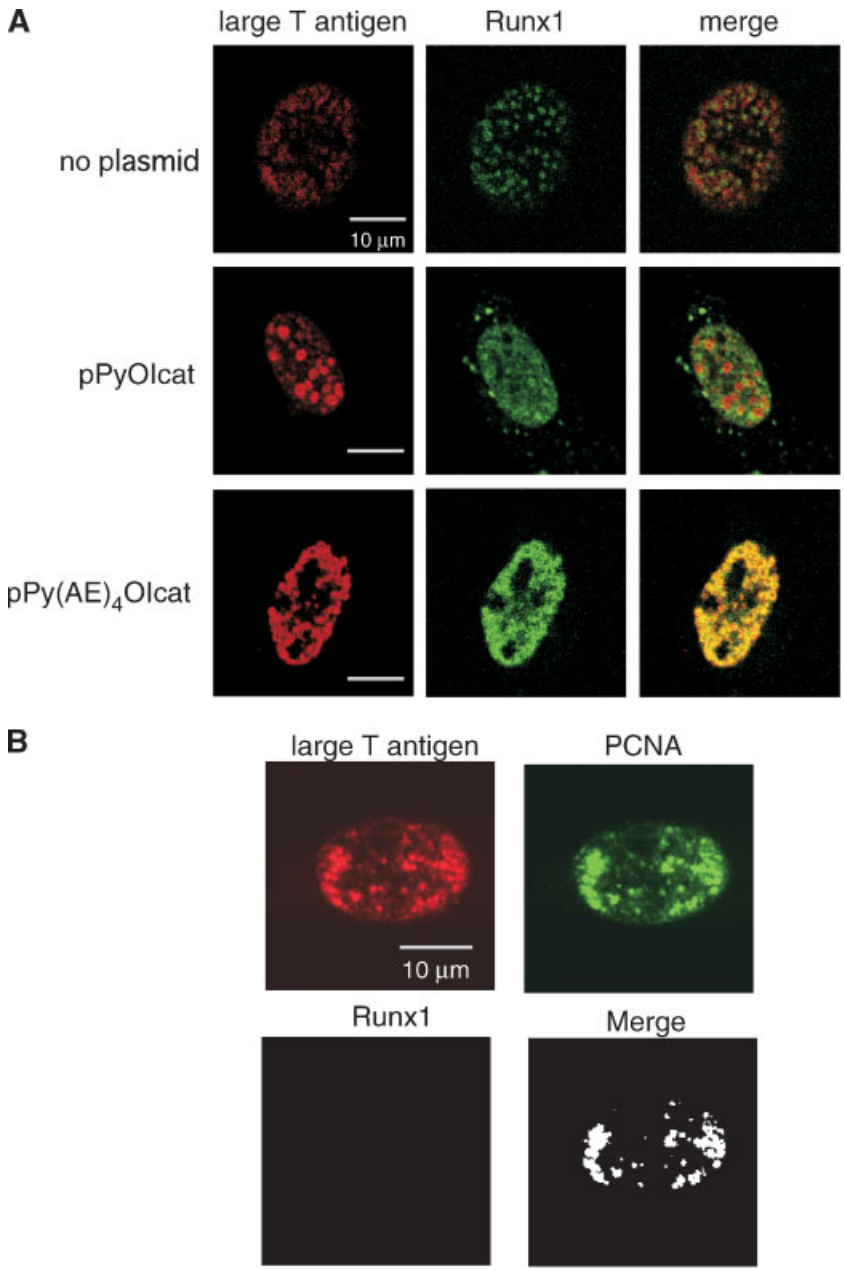

Fig. 4. The Runx1 foci co-localize with the large T antigen and PCNA foci only when Runx1-dependent Py DNA replication is taking place. **A**: Localizations of large T antigen and Runx1. NIH3T3 cells were co-transfected with large T antigen (1.5 μg) and HA-tagged Runx1 (1.0 μg) expression plasmids in the presence or absence of Py core origin-containing plasmids (0.1 μg). $pPy(AE)_4OIcat$ contains Runx-binding sites, unlike pPyOIcat. The localization of large T antigen (red, Cy3) and Runx1 (green, FITC) was examined as described in Figure 2. **B**: Localization of Runx1, large T antigen, and PCNA. The localizations of large T antigen (red, Cy3), PCNA (green, FITC), and PCNA (blue, Cy5) were analyzed simultaneously. The co-localization of the three proteins generates a white signal in the merged panel.

mutation in RAD disrupted the interaction [Chen et al., 1998]. Although the nature of this factor is not yet clear, it could involve remodeling of the chromatin structure at the core origin for the efficient binding of large T antigen. Indeed, AML1 is shown to interact with p300 and CBP histone acetyltransferases that supposed to change chromatin structure through histone acetylation [Kitabayashi et al., 1998].

Targeting of large T antigen to replication factories is reasonable strategy for viral replica-

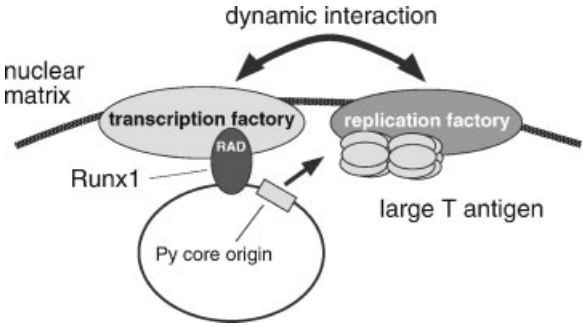

Fig. 5. Model of Runx1 stimulation of Py DNA replication. Large T antigen targets to the replication factory on the nuclear matrix but can not bind Py origin by itself. Runx1 first binds to its binding sites close to Py replication origin and tether the origin to the transcription factory on the nuclear matrix through the nuclear matix-targeting activity of RAD. This tethering would help the orgin to encounter large T antigen at the replication factories, since both factories are dynamically co-organized during S-phase. For detail discussion, see text.

tion that utilizes cellular DNA replication machinery. Some proteins functioning at the replication fork, such as DNA ligase I and replication factor C (RF-C), has been shown to have replication factory targeting motif, which shares homology to PCNA-binding motif [Montecucco et al., 1998]. However, Py large T antigen does not have such motives. Therefore, the targeting mechanism of large T antigen is not clear at this stage. Recently, SV40 large T antigen was shown to interact with nucleolin [Seinsoth et al., 2003], which is a component of the nuclear matrix [Dickinson and Kohwi-Shigematsu, 1995; Gotzmann et al., 1997] and is thought to be involved in DNA replication [Xu et al., 2001]. Since Py large T antigen is very similar to the SV40 large T antigen, it seems likely that nucleolin will also play a role in its targeting to replication factories.

We found that only a small portion of the Py origin-containing reporter plasmid present in the Triton-insoluble fraction actually replicated (Fig. 2). Most of the transfected reporter plasmid was probably nonspecifically localized to the insoluble fraction, since even the pHSG398 control plasmid, which neither harbors the core origin nor Runx1-binding sites, was found in both soluble and insoluble fractions. We speculate that only a small fraction of the transfected reporter plasmids can localize to replication factories in a manner that permits their efficient replication. Supporting this, we previously observed similar inefficient replication of transfected ARS plasmids in budding yeast [Kohzaki et al., 1999].

The existence of replication factories implies the existence of mechanisms that regulate the recruitment of origins to replication factories [Cook, 1999]. In somatic mammals, ORC1, a component of the six subunit cellular initiator complex origin recognition complex (ORC), has been shown to not only associate with other ORC subunits that stably bind to origins but also to bind to the nuclear matrix in late G_1 phase [Ohta et al., 2003]. This suggests that origins associate in a dynamic fashion with the nuclear matrix [Djeliova et al., 2001]. Matrix attachment regions, in the form of nuclear matrix attachment sequences, are a common feature of replication origins of mammals examined to date. However, the importance of such regions for origin activity remains unclear [DePamphilis, 2000; Anachkova et al., 2005]. Our results suggest that nuclear matrix-binding transcription factors that bind close to origins may help regulate origins in a dynamic fashion. Indeed, eukaryotic origins so far analyzed contains transcription factor-binding sites or localized promoter or regulatory region of transcription [Kohzaki and Murakami, 2005]. For example, transcription factors, Myb and E2F, are implicated in the regulation of *Drosophila* chorion gene amplification [Beall et al., 2002]. Since the transcription factors are the final nuclear targets of the signal transduction, it is attractive to speculate that they regulate DNA replication in response to extra/intracellular signals. Further analysis of the cellular origins will clarify this issue. In addition, another important way transcription factors might regulate nuclear processes is to recruit target DNA to specialized nuclear compartments.

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