Multiple Mechanisms Contribute to the Activation of RNA Polymerase III Transcription in Cells Transformed by Papovaviruses

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RNA polymerase (pol) III transcription is abnormally active in fibroblasts transformed by polyomavirus (Py) or simian virus 40 (SV40). Several distinct mechanisms contribute to this effect. In untransformed fibroblasts, the basal pol III transcription factor (TF) IIIB is repressed through association with the retinoblastoma protein RB; this restraint is overcome by large T antigens of Py and SV40. Furthermore, cells transformed by these papovaviruses overexpress the BDP1 subunit of TFIIIB, at both the protein and mRNA levels. Despite the overexpression of BDP1, the abundance of the other TFIIIB components is unperturbed following papovavirus transformation. In contrast, mRNAs encoding all five subunits of the basal factor TFIIIC2 are found at elevated levels in fibroblasts transformed by Py or SV40. Thus, both papovaviruses stimulate pol III transcription by boosting production of selected components of the basal machinery. Py differs from SV40 in encoding a highly oncogenic middle T antigen that localizes outside the nucleus and activates several signal transduction pathways. Middle T can serve as a potent activator of a pol III reporter in transfected cells. Several distinct mechanisms therefore contribute to the high levels of pol III transcription that accompany transformation by Py and SV40.

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The abbreviations used are: pol, RNA polymerase; ARPP, acidic ribosomal phosphoprotein; BDP1, B double prime 1; BRF1, TFIIIB-related factor 1; EMSA, electrophoretic mobility shift assay; LT, large T antigen; MT, middle T antigen; Py, polyomavirus; RT, reverse transcription; SV40, simian virus 40; TBP, TATA-binding protein; TF, transcription factor; snRNA, small nuclear RNA; CAT, chloramphenicol acetyltransferase.

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cific, because no increase was observed in mRNAs encoding glyceraldehyde-3-phosphate dehydrogenase or the TFIIIB subunit BRF1 (14). These SV40-transformed cell lines therefore deregulate two key components of the class III machinery to achieve high rates of pol III transcription; this involves release from repression in the case of TFIIIB, whereas TFIIIC2 hyperactivity reflects its increased production.

Although two subunits of TFIIIC2 were shown to be overexpressed in SV3T3 cells (14), its other three subunits had not been isolated at that time and so were not examined. As their sequences are now available (25, 26), we are able here to confirm that each of their mRNAs shows a similar response to SV40 transformation, allowing a coordinated induction. Furthermore, that correct isolation (27) of the remaining human TFIIIB subunit BDP1 (previously referred to as B') has allowed us to also examine if this polypeptide responds to transformation. Unlike the other components of TFIIIB, we find that BDP1 is overproduced at both the mRNA and protein levels in SV3T3 cells. We have extended these observations to include polyomavirus (Py), a second member of the papovavirus family that causes a variety of tumors in newborn mice (28). We show that Py-transformed fibroblasts resemble SV3T3 cells in that they overexpress BDP1 and all five subunits of TFIIIC2 and that they use large T antigen to release TFIIIB from repression by RB. Whereas SV40 just has large T and small t antigens, Py also makes a middle T antigen which is essential for its transformation (28). Although Py middle T is not found in the nucleus, we show that it too can activate pol III transcription in vivo, probably via one or more signaling cascades. The class III machinery is therefore deregulated through several distinct mechanisms in fibroblasts transformed by SV40 and Py, allowing these papovaviruses to achieve the high rates of pol III activity that are necessary for cells to sustain rapid growth.

EXPERIMENTAL PROCEDURES

Cell Culture—SV3T3 lines were generated by infection of Balb/c 3T3 A31 cells with SV40 of the wt830 strain and were selected by focus formation in low serum (29). Py3T3 and Pytsa3T3 are lines of A31 cells transformed by wild-type Py and tsA mutant virus, respectively (2). All cell lines were grown in Dulbecco’s modified Eagle’s medium (Invitrogen) supplemented with 10% fetal calf serum, 100 units/ml penicillin, and 100 μg/ml streptomycin, and were harvested when subconfluent.

RT-PCR Analysis—RNA was extracted from cells using TRI reagent (Sigma), according to the manufacturer’s specifications. All primers have been described previously (9), apart from those involving BDP1. PCR of BDP1 used primers 5’-GCTGATAGAGACTCTCTC-3’ and 5’-CCAGAGAAGAATCTTCTC-3’ to give a 293-bp product and the following amplification conditions: 95 °C for 2 min; 35 cycles of 95 °C for 1 min, 56 °C for 1 min, and 72 °C for 1 min; and 72 °C for 5 min. Amplification was carried out over a linear range using conditions described previously (9), except for the inclusion of 1.8 μCi of [α-32P]dCTP (10 mCi/ml, 3000 Ci/mmol). Reaction products were resolved on a 7% polyacrylamide gel containing 7 M urea and 0.5× TBE. Radioactivity was visualized by autoradiography and quantitated by phosphorimaging (Fuji Bas 1000).

Plasmids—Details of pol III templates pVA1, Mctel1, pGlu6, E2-160, and pTBr1 have all been published (30, 31). pCAT (Promega) contains the CAT gene driven by the SV40 promoter and enhancer. Expression constructs pSV-LT, pSV-MT, and pSV-NG95 have been described (32, 33).

Transfection Assays—Cell lines were transiently transfected using Superfect (Qiagen), according to the manufacturer’s specifications. Total RNA was extracted 48 h after transfection using TRI reagent (Sigma), according to the manufacturer’s instructions. It was then analyzed by primer extension using both VA1-specific (5’-CACCGCGCGGCTA- ACCGCGATG-3’) and CAT-specific (5’-CCAGAGAAGAATCTTCTC- 3’) labeled primers. Primer extension reactions were conducted as previously described (18).

Transcription—Pol III transcription was carried out as previously described (34), except that pBR322 was not included and the incubations were for 60 min at 30 °C.

Preparation of Extracts—Whole cell extracts were prepared using the method described by White et al. (31).

Immunoprecipitation—Extract (150 μg) was incubated at 4 °C on an orbital shaker with 20 μl of protein A-Sepharose beads carrying equivalent amounts of prebound IgG. Samples were then pelleted, supernatants were removed, and the beads were washed five times with 150 μl of LDB buffer (20 mM HEPES-KOH, pH 7.9, 17% glycerol, 100 mM KCl, 12 mM MgCl2, 0.1 mM EDTA, 2 mM dithiothreitol). The bound material was analyzed by Western blotting.

Antibodies and Western Blotting—Antibodies used were C-15 (Santa Cruz) and G99-549 (Pharmingen) against RB, M-19 (Santa Cruz) against TAF48, C18 (Santa Cruz) against TFIIIB, 330 and 128 against BRF1 (35, 36), SL30 against TBP (37), clone 46 (Transduction Laboratory) against TFIIIC110, and 1404 against TFIIIC2 (38). Antibodies 2663 against BDP1 was raised by immunizing rabbits with synthetic peptide CSYDRYRIYKAQKLRE (human BDP1 residues 139–152 (27)) coupled to keyhole limpet hemocyanin. Antibodies 1898 against TFIIIC90 was raised by immunizing rabbits with synthetic peptides MNTADQARVGPADDGC and GMGNADDEQEGGTC (human TFIIIC90 residues 1–16 and 613–626 (26), respectively) coupled to keyhole limpet hemocyanin. Western immunoblot analysis was performed as previously described (31).

RESULTS

Activation of Pol III Transcription by Papovaviruses

Class III Gene Expression Is Elevated in Py-transformed 3T3 Cells Relative to the Parental 3T3 A31 Cells—Previous studies have shown that pol III transcripts are overexpressed in a large number of rat, mouse, and hamster fibroblast cell lines that have been transformed with Py, relative to their untransformed parental lines (2, 39, 40). Nuclear run-on assays revealed that this is because of an increased rate of pol III transcription (40). However, these earlier studies did not investigate the molecular basis of the induction. To do this, we have concentrated on the murine line Py3T3, a Py-transformed derivative of A31 cells, an immortalized Balb/c 3T3 line (2). This was chosen to allow direct comparison with our previous data on SV3T3 cells, which were generated from the same A31 parental line by SV40 transformation (29).

To confirm that the Py3T3 cells display a general deregulation of class III genes, RT-PCR reactions were carried out over a linear range with template cDNAs generated using RNA extracted from the Py3T3 and A31 3T3 lines. The level of tRNA27 and U6 mRNA was clearly elevated in the Py3T3 cells (Fig. 1). This was also true for tRNA23ss (data not shown). In contrast, little or no change was detected in the levels of mRNA encoding acidic ribosomal phosphoprotein P0 (ARP5), which is synthesized by pol II. In the case of the tRNAs, our primers hybridize to the intron sequences of short-lived primary transcripts; because these tRNA precursors are processed very rapidly, their levels in a cell reflect the rate of ongoing transcription (41). Our data therefore suggest that pol III activity is elevated specifically in the Py3T3 line relative to the untransformed parental cells.

Large T Antigen Activates TFIIIB and Dissociates BRF1 from RB in Py3T3 Cells—In untransformed fibroblasts, TFIIIB is bound and repressed by RB (19). The large T antigen of SV40 was shown to overcome this repressive influence of RB on TFIIIB (14). Because Py also encodes a large T antigen that can bind and neutralize RB (42–44), we investigated whether this viral product can activate TFIIIB by releasing it from RB.

Whole cell extracts were prepared in parallel from 3T3 and Py3T3 cells. As expected, the transformed cell extracts transcribed a range of pol III templates more actively than the matched 3T3 extracts (Fig. 2A). Complementation experiments were conducted to compare directly the TFIIIB activity in the extracts. These assays exploit the differential sensitivity of pol III factors to inactivation by mild heat treatment. When an extract is heated at 47 °C for 15 min, TFIIIC and TBP are inactivated whereas the other components of the pol III machinery are not compromised; the activity of TFIIIB in an
unfractionated extract can therefore be assayed by heating the extract and then measuring its ability to reconstitute transcription when added to a complementing fraction containing TFIIIC, TBP, and pol III (14, 19, 31, 35, 36). This revealed that TFIIIB activity is elevated 6-fold in extracts of the Py3T3 line (Fig. 2B). To test whether large T antigen is responsible for this increase, we used Pytsa3T3 cells, a line generated by transforming 3T3 A31 cells using a Py mutant in which large T is inactivated specifically (2). In contrast to the extracts of cells transformed with wild-type virus, Pytsa3T3 cell extracts showed only a 1.3-fold increase in TFIIIB activity (Fig. 2B). This suggests that large T antigen is primarily responsible for the elevated TFIIIB activity observed in Py3T3 cells.

Because the above experiments implicate Py large T in the deregulation of TFIIIB and this T antigen is known to target RB, we carried out immunoprecipitation experiments to investigate how the interaction between RB and TFIIIB responds to Py transformation. As shown previously (14, 19, 20, 45, 46), the BRF1 subunit of TFIIIB can be coimmunoprecipitated using antibody against RB (Fig. 3A, upper panel). This reflects a specific interaction with RB, because BRF1 is not detected in immunoprecipitates obtained using antibody against TAF1, which serves as a negative control. Although similar amounts of RB are immunoprecipitated from the two cell types (lower panel), the amount of bound BRF1 is substantially reduced in the Py3T3 cells when compared with 3T3. Western blotting of whole cell extracts shows that the reduced interaction between RB and BRF1 cannot be explained by a decrease in the level of either of these components (Fig. 3B). We also detect no change in the abundance of TBP.

The results above show that the proportion of BRF1 bound to RB is significantly reduced following transformation by Py. The most likely explanation is that much of the RB in Py3T3 cells is bound and neutralized by the viral large T antigen. Indeed, immunoprecipitations confirmed that large T antigen associates with RB in the Py3T3 cell extracts. To test directly whether Py large T can activate pol III transcription in vivo, we carried out transient transfections. 3T3 cells were transfected with the adenovirus VA1 gene, as a pol III reporter, and the CAT gene driven by the SV40 early promoter, as an internal pol II control. Cotransfection with a vector encoding Py large T antigen resulted in a dramatic, specific, and dose-dependent activation of VA1 expression (Fig. 4A). As a negative control, we used a mutant form of Py middle T, which had no effect on the pol III reporter. After normalization against the SV40-CAT internal control, the Py large T antigen was found to stimulate VA1 gene expression by over 50-fold in this assay (Fig. 4B).

The BDP1 Subunit of TFIIIB Is Overexpressed in Py3T3 Cells—TFIIIB contains three essential subunits, TBP, BRF1, and BDP1 (23, 24). Although the levels of TBP and BRF1 are similar in 3T3 and Py3T3 cell extracts (Fig. 3B), we found that

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2 Z. A. Felton-Edkins, unpublished data.
the BDP1 protein is significantly more abundant after Py transformation (Fig. 5A, upper panel). This overexpression does not require large T antigen, as it is seen with the Pytsa mutant as well as the wild-type virus. The effect is specific, because equal amounts of TFIIIB are detected in the three cell types (Fig. 5A, lower panel). It was confirmed using an alternative antibody against BDP1. To find out if overproduction of BDP1 is a more general feature of papovavirus-transformed fibroblasts, we carried out similar blots with SV40-transformed SV3T3 Cl38 and SV3T3 Cl49, which were found to produce abnormally high levels of BDP1 (Fig. 5B). As with Py3T3 cells, the BRF1 and TBP levels are normal in these SV3T3 lines (14). Thus, transformation by both Py and SV40 can induce a selective increase in the abundance of a specific subunit of TFIIIB.

RT-PCR assays showed that in each case the increase in BDP1 was associated with a selective increase in the mRNA encoding BDP1 (Fig. 5, C and D).

**TFIIC2 Is Overexpressed in Py3T3 Cells**—Overexpression of BDP1 in transformed cells has not been reported previously. However, SV3T3 cells have been shown to overexpress the two largest subunits of TFIIC2, TFIIC220 and TFIIC110 (also called TFIICα and TFIICβ, respectively), at both the protein and mRNA levels (14). Elevated expression of TFIIC220 and TFIIC110 was also detected in Py3T3 cells, as revealed by Western immunoblotting with antibodies against BDP1 (upper panel), BRF1 (middle panel), and TBP (lower panel). The BDP1 protein is significantly more abundant after Py transformation (Fig. 5A, upper panel). This overexpression does not require large T antigen, as it is seen with the Pytsa mutant as well as the wild-type virus. The effect is specific, because equal amounts of TFIIB are detected in the three cell types (Fig. 5A, lower panel). It was confirmed using an alternative antibody against BDP1. To find out if overproduction of BDP1 is a more general feature of papovavirus-transformed fibroblasts, we carried out similar blots with SV40-transformed lines. Both SV3T3 Cl38 and SV3T3 Cl49 were found to produce abnormally high levels of BDP1 (Fig. 5B). As with Py3T3 cells, the BRF1 and TBP levels are normal in these SV3T3 lines (14). Thus, transformation by both Py and SV40 can induce a selective increase in the abundance of a specific subunit of TFIIB.

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mRNA, which was only 2-fold elevated. It is important to note that the overexpression of TFIIIC2 in the transformed cell lines is not because of their higher rates of proliferation. Previous studies have demonstrated that there is no difference in expression of any of the five TFIIIC2 subunit mRNAs when fibroblasts that are actively proliferating are compared with those that have been growth-arrested by serum deprivation (9, 46). The elevated expression of these transcripts therefore appears to be a response to transformation per se, rather than to elevated proliferation.

In SV3T3 cells, the overproduction of TFIIIC2 subunits is accompanied by increased amounts of TFIIIC2 activity, as revealed by EMSA (4, 14). Although the overall induction of TFIIIC2 is lower following Py transformation, EMSA nevertheless revealed a reproducible increase in Py3T3 cell extracts (Fig. 8A). Quantitation revealed that TFIIIC2 activity is typically ~1.8-fold greater (Fig. 8B). This is consistent with the 1.5–2.5-fold overexpression of TFIIIC2 subunits seen in Py-transformed cells (Figs. 6 and 7). Supershift experiments confirmed that TFIIIC2 is responsible for the observed complex formed with the B-block promoter sequence probe. Thus, the complex is supershifted by an antiseraum against TFIIIC90, but not by the corresponding preimmune serum (Fig. 8C). It can also be competed specifically using unlabeled B-block oligonucleotide.2 We conclude that Py resembles SV40 in being able to raise the amount of active TFIIIC2 during transformation of 3T3 fibroblasts.

**Middle T Antigen Contributes to the Activation of Pol III Transcription in Py3T3 Cells**—The increase in TFIIIC2 seen in Py3T3 cells is also observed in the Py3a3T3 line where large T antigen is defective (Fig. 8). Similarly, the cells transformed in the absence of functional large T overexpress TFIIIC2 mRNAs at comparable levels to the wild-type Py3T3 line (Fig. 6). Thus, although large T antigen is primarily responsible in this system for activating TFIIIB (Fig. 2B), it is not required for the observed increase in TFIIIC2 levels.

To compare the relative contributions of large T-dependent and large T-independent mechanisms to the deregulation of pol III activity in Py-transformed fibroblasts, we compared overall expression levels in Py3T3 and Py3a3T3 cells (Fig. 9). After normalization against the ARPP P0 internal control, pol III transcripts were found to be ~11-fold higher in Py3T3 relative to the parental 3T3 line. In contrast, the Py3a3T3 derivative lacking large T showed a ~4-fold activation. This confirms that large T plays a major part in activating the pol III machinery in vivo, consistent with its requirement for derepressing TFIIIB and its ability to induce a transfected VA1 gene (Fig. 4). However, it also shows that large T-independent effects make a significant contribution to the induction of pol III transcription following Py transformation.

In contrast to SV40, Py encodes a middle T antigen that is essential for it to transform cultured cells or cause tumors in mice (28, 49). Indeed, middle T alone is sufficient to transform established cell lines, although the immortalizing function of large T is also required for transforming primary fibroblasts (43, 44, 50). Because of the pivotal role played by middle T in the oncogenicity of Py, we tested whether it is capable on its own of activating pol III transcription. 3T3 cells were transfected with the VA1 gene as pol III reporter and SV40-CAT as an internal pol II control. Cotransfection with a vector encoding Py middle T antigen resulted in a ~2-fold activation. This confirms that middle T is also required for transforming primary fibroblasts (43, 44, 50). Because of the pivotal role played by middle T in the oncogenicity of Py, we tested whether it is capable on its own of activating pol III transcription. 3T3 cells were transfected with the VA1 gene as pol III reporter and SV40-CAT as an internal pol II control. Cotransfection with a vector encoding Py middle T antigen resulted in a ~2-fold activation. This confirms that middle T is also required for transforming primary fibroblasts (43, 44, 50). Because of the pivotal role played by middle T in the oncogenicity of Py, we tested whether it is capable on its own of activating pol III transcription. 3T3 cells were transfected with the VA1 gene as pol III reporter and SV40-CAT as an internal pol II control. Cotransfection with a vector encoding Py middle T antigen resulted in a ~2-fold activation. This confirms that middle T is also required for transforming primary fibroblasts (43, 44, 50). Because of the pivotal role played by middle T in the oncogenicity of Py, we tested whether it is capable on its own of activating pol III transcription. 3T3 cells were transfected with the VA1 gene as pol III reporter and SV40-CAT as an internal pol II control. Cotransfection with a vector encoding Py middle T antigen resulted in a ~2-fold activation. This confirms that middle T is also required for transforming primary fibroblasts (43, 44, 50). Because of the pivotal role played by middle T in the oncogenicity of Py, we tested whether it is capable on its own of activating pol III transcription. 3T3 cells were transfected with the VA1 gene as pol III reporter and SV40-CAT as an internal pol II control. Cotransfection with a vector encoding Py middle T antigen resulted in a ~2-fold activation. This confirms that middle T is also required for transforming primary fibroblasts (43, 44, 50).
middle T antigen was found reproducibly to stimulate VA1 gene expression by 40–50-fold in this assay (Fig. 10B). This oncoprotein can therefore serve as a very potent inducer of pol III transcription in vivo, despite the fact that it is situated at membranes outside of the nucleus (51, 52). Its ability to achieve this activation is likely to result from its well-documented capacity to stimulate a variety of signal transduction pathways (49, 53). This possibility is supported by the failure of NG59 to increase VA1 expression, because this substitution mutant (Asp-179 replaced by Ile-Asn) is unable to activate the signaling cascades that respond to wild-type middle T (32). These data suggest that middle T is likely to play a significant part in deregulating pol III in Py-transformed cells.

**DISCUSSION**

It has long been recognized that SV40 transformation results in a marked increase in the expression of pol III transcripts (2–4, 10). Recent work identified two distinct mechanisms that contribute to this effect (14). A major restraint on pol III transcription in untransformed fibroblasts is provided by RB and its relatives p107 and p130, which bind and repress TFIIIB (14, 19, 22, 45, 46). The large T antigen of SV40 can bind and neutralize RB, p107, and p130 and thereby release TFIIIB from
this control, allowing a marked increase in its transcriptional activity (14). This effect can explain the speed with which pol III transcription increases when temperature-sensitive large T antigen is transferred to the permissive temperature; synthesis of 7SK RNA rises by 7-fold within 30 min, despite a slowing of proliferation (11). A second and apparently unrelated mechanism that accompanies SV40 transformation involves the overexpression of TFIIIC2; EMSAs showed elevated TFIIIC2 activity, whereas RT-PCR and Western blotting revealed that this correlates with elevated levels of TFIIIC220 and TFIIIC110 mRNA and protein (4, 14). The current study has confirmed that the remaining three subunits of TFIIIC2 are also overexpressed in SV40-transformed cells, at both the mRNA and protein levels. Although this might have been anticipated, because the five subunits are assumed to function in a stoichiometric complex, one study found that TFIIIC110 is induced selectively by adenovirus infection (47). In contrast to the increases in TFIIIC2 subunits, there is little or no change in the levels of the TBP and BRF1 components of TFIIIB (14). Because TFIIIB is also believed to be a stoichiometric complex, we had expected that its third essential subunit would also remain constant following SV40 transformation. However, we discovered that BDP1 is clearly overexpressed at both the mRNA and protein levels in SV3T3 cells, providing an additional and unanticipated instance of how the pol III machinery can be affected by a virus. Transformation by SV40 therefore involves at least three distinct changes to the basal pol III factors, which combine to allow unusually high transcription of class III genes.

We have extended these observations by examining the effects of Py, another papovavirus that differs from SV40 in several important ways (54). We demonstrate that Py large T releases TFIIIB from repression by RB and that Py-transformed fibroblasts overexpress mRNAs encoding all five subunits of TFIIIC2, as well as the BDP1 subunit of TFIIIB, but not TBP or BRF1. Thus, these three mechanisms of deregulation are shared by different branches of the papovavirus family. Other types of DNA tumor virus also activate pol III transcription and it will be interesting to assess the extent to which they employ similar deregulatory mechanisms. RB is a common target for transforming viruses and we have demonstrated previously that both adenovirus and human papillomavirus can stimulate pol III activity by using oncoproteins to neutralize RB (14, 18, 45). Indeed, inactivation of RB may be the most common mechanism for inducing pol III transcription in transformed cells (55). Induction of TFIIIC2 may also prove to be a strategy that is commonly used by viruses, because early template commitment assays provided evidence that the concentration of this factor increases when HeLa cells are infected with adenovirus (56). A more recent study, however, found that
TFIIIC220 is not induced in adenovirus-infected HeLa cells, although TFIIIC110 levels increase markedly; the remaining TFIIIC2 subunits and the components of TFIIIB were not examined in that study (47). The latter report clearly differs from the effects we have observed, but TFIIIC220 levels may be very high already in uninfected HeLa cells, which are transformed by human papillomavirus; furthermore, infection may elicit a different TFIIIC2 response to transformation.

The overexpression of TFIIIC2 is a clinically relevant phenomenon, because it has been found to occur in human cancers. Thus, a study of nine ovarian epithelial carcinomas revealed abnormally high TFIIIC2 activity in each of the tumors when compared with untransformed ovarian tissue from the same individuals (9). This effect correlated with a specific increase in the levels of all five mRNAs encoding the subunits of TFIIIC2 (9). Because ovarian cancer is not believed to be associated with tumor viruses (57), it seems that TFIIIC2 expression can respond to distinct types of oncogenic signal. The overexpression of TFIIIC2 is a clinically relevant phenomenon, because it has been found to occur in human cancers. Thus, a study of nine ovarian epithelial carcinomas revealed abnormally high TFIIIC2 activity in each of the tumors when compared with untransformed ovarian tissue from the same individuals (9). This effect correlated with a specific increase in the levels of all five mRNAs encoding the subunits of TFIIIC2 (9). Because ovarian cancer is not believed to be associated with tumor viruses (57), it seems that TFIIIC2 expression can respond to distinct types of oncogenic signal. The overexpression of TFIIIC2 is a clinically relevant phenomenon, because it has been found to occur in human cancers. Thus, a study of nine ovarian epithelial carcinomas revealed abnormally high TFIIIC2 activity in each of the tumors when compared with untransformed ovarian tissue from the same individuals (9). This effect correlated with a specific increase in the levels of all five mRNAs encoding the subunits of TFIIIC2 (9). Because ovarian cancer is not believed to be associated with tumor viruses (57), it seems that TFIIIC2 expression can respond to distinct types of oncogenic signal.

The use of papovavirus-transformed cell lines first uncovered this feature of pol III regulation (4, 14) and it is gratifying that these model systems have proved again to be relevant to human disease. We intend to employ them to investigate further the mechanisms responsible for inducing TFIIIC2 during carcinogenesis.

In the three types of tumor cell we have analyzed to date (SV3T3, Py3T3, and ovarian carcinomas), the five transcripts encoding the components of TFIIIC2 are all induced together. This coordinate induction under distinct circumstances sug-
gests that the genes encoding these subunits might have common promoter or enhancer sequences that allow their coregulation. This seems logical because the five subunits are believed to function stoichiometrically. However, studies with HeLa extracts have suggested the existence of an inactive TFIIIC2 complex that specifically lacks the TFIIIC110 subunit (47, 58, 59). This lead to a model in which the selective induction of TFIIIC110 might convert pre-existing inactive complexes into functionally competent TFIIIC2 (47, 58, 59). In regard to this model, it is notable that TFIIIC110 is more strongly induced by papovavirus transformation than the other components of the complex, especially in the SV3T3 cells (14). However, this was not the case in ovarian cancers (9).

The induction of BDP1 we report here has not been observed previously, principally because mammalian BDP1 has only been identified recently (27). It is somewhat surprising, because the TBP and BRF1 components of TFIIIB are not overexpressed in SV3T3 or Py3T3 cells. Nevertheless, the level of BDP1 mRNA and protein is clearly elevated in the papovavirus-transformed lines we have examined. As in yeast, mammalian BDP1 has a relatively low affinity for the TBP/BRF1 subcomplex (27). Increasing the level of BDP1 might promote its assembly into functional TFIIIB complexes by mass action. However, direct assays show that TFIIIB activity is not substantially elevated in Pytsa3T3 cells, despite the fact that this line produces BDP1 at a comparable level to Py3T3 cells; this indicates that TFIIIB activation in these lines is primarily large T-dependent, reflecting neutralization of RB, and is not principally caused by overexpression of BDP1. Thus, it appears that elevated BDP1 levels may not be making a major contribution to the deregulation of pol III transcription that accompanies Py transformation of fibroblasts. However, the relative importance of the different pathways used by Py to act on pol III may vary according to cell type. For example, induction of BDP1 might be much more important in the endotheliomas that Py causes in mice. Its impact might also vary between different pol III promoters.

Our study revealed that Py middle T antigen can strongly activate a pol III reporter in vivo (Fig. 10). Middle T is generated by alternative splicing of the viral early transcript and has no equivalent in SV40 (54). Nevertheless, it is the principal oncoprotein of Py, being sufficient to transform immortalized cells (28, 43, 44, 49, 50). It achieves this through association with signal transducers, such as members of the Src family, phosphatidylinositol 3-kinase, and the SHC protein that activates the Ras pathway (see Refs. 32 and 53 and references therein). Because middle T is located outside the nucleus, we assume that it stimulates pol III transcription through its action on signaling cascades. Indeed, we have evidence that the activation of pol III in Py3T3 cells can be partially blocked using specific kinase inhibitors. We are currently investigating the pathways involved and how these impact on the pol III machinery. The action of middle T is reminiscent of the situation with the X oncoprotein of hepatitis B virus, which stimulates pol III transcription by activating the Ras/Raf-1 signal transduction cascade (6).

The region of the Py genome that encodes middle T is poorly conserved with SV40 (54). The equivalent SV40 sequence codes for a large T epitope that binds and inactivates p53, a function not performed by any Py product (60–62). Because p53 has been shown to bind and inactivate TFIIIB (36, 63), release from p53 repression may provide yet another mechanism that helps to deregulate pol III transcription in some types of SV40-transformed cells. A physical interaction between TFIIIB and SV40 large T has also been reported (13). The contribution of these mechanisms toward pol III activation has yet to be established. Nevertheless, it seems clear now that papovavirus transformation can impact on the pol III machinery in a number of ways. The targeting of both TFIIIB and TFIIIC2 may be important to maximize pol III transcription. Experiments with synchronized cell populations revealed that alternative pol III factors can be limiting during different phases of the cell cycle (64). Whereas TFIIIC activity limits the rate of VA1 expression in extracts of S or G2 phase cells, TFIIIB is the limiting factor in extracts of cells harvested during G0 or early G1 (46, 64). Stimulation of TFIIIB or TFIIIC alone might therefore only influence the transcriptional output during a restricted interval of the cell cycle. Activating both TFIIIB and TFIIIC may allow papovavirus-transformed cells to sustain elevated rates of pol III transcription throughout interphase, which may be a prerequisite for rapid growth. What is surprising, perhaps, is the diversity of deregulatory mechanisms that appear to contribute to this end.

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