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DNA-Mediated Transfer of an RNA Polymerase II Gene: Reversion of the Temperature-Sensitive Hamster Cell Cycle Mutant TsAF8 by Mammalian DNA

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Treatment of the TsAF8 temperature-sensitive (TS) mutant of Syrian hamster BHK-21 cells, with calcium phosphate precipitates of genomic TS^+ DNAs from a variety of mammalian cell lines permitted the selection of TS^+ colonies at 40°C. TS^+ transformation events were distinguished from spontaneous TS^+ reversions in experiments in which α-amanitin-sensitive (Ama^s) TS^+ DNA was used to transform an Ama^R derivative of TsAF8 cells and Ama^R TS^+ DNA was used to transform Ama^s TsAF8 cells. In each case it was possible to demonstrate the unselected acquisition of the appropriate Ama^s or Ama^R phenotype with the selected TS^+ allele. Each of these TS^+ transformed cell lines when grown at 40°C contained an RNA polymerase II activity with a sensitivity to inhibition by α-amanitin characteristic of the particular DNA used to transform the TS cells, whereas at 34°C the same cells contained a mixture of Ama^R and Ama^s polymerase II activities. Together, these data provide convincing evidence that the RNA polymerase II gene determining sensitivity to inhibition by α-amanitin can be transferred to TsAF8 cells and that the TS defect in TsAF8 is a polymerase II mutation.

An understanding of the biochemical mechanisms which regulate both the activity and the selectivity of RNA polymerase (RNA nucleotidyltransferase) in mammalian cells should be facilitated by the availability of mutant cell lines containing altered RNA polymerase activities. Mutations affecting the activity of RNA polymerase II in several cultured cell lines have already been described. These mutations are of three types: resistance to the RNA polymerase II inhibitor α-amanitin (4, 12, 23), conditional lethal temperature-sensitive (TS) mutations in polymerase II (11), and several second-site mutations that revert the TS effects on growth of a Chinese hamster ovary (CHO) TS polymerase II mutation (22). With this limited number of mutants, it is clear that only a modest beginning has been made in genetically defining the various components of the transcription complex in animal cells; indeed, both the Ama^R and the TS mutations are in the same complementation group (22). Although these mutations have been of some use in demonstrating the regulated synthesis of polymerase II subunit polypeptides (7, 8, 24), there exists a need for both better characterization of the existing mutations and the isolation of a wider spectrum of mutations affecting RNA synthesis in animal cells. Such goals could be more readily realized were the gene(s) for eucaryotic RNA polymerase II isolated.

Gene isolation has, for the most part, been accomplished to date by using DNA cloning of abundant mRNA species, but the isolation of genes represented only infrequently in mRNA populations, such as those for RNA polymerase II polypeptides, requires different experimental approaches. DNA transfer of genes expressing selectable phenotypes to appropriate recipient cells, followed by recombinant DNA screening to rescue transforming DNA, has been shown to be a successful method for the isolation of the genes coding for less abundant enzymes, such as thymidine kinase (19) and adenine phosphoribosyltransferase (10). However, despite the codominant inheritance of α-amanitin resistance, gene transfer of the Ama^R phenotype has proven difficult in several laboratories. In this study we show that with an alternative approach, involving transfer of the dominant wild-type TS^+ gene to the TS BHK-21 cell line TsAF8, it is possible to establish that the TS defect in TsAF8 is a polymerase II mutation and that the gene determining sensitivity to α-amanitin is the TS^+ polymerase II gene.

MATERIALS AND METHODS

Cells and cell culture. BHK-21/13 cells and those of the BHK-21/13 mutant TsAF8, derived by Meiss and

Basilico (17), were obtained from Renato Baserga (Temple University, Philadelphia, Pa.). The CHO wild-type and mutant Ama1 cells have been described previously (12), as has the human diploid fibroblast mutant strain Ama1070 (2). BHK and HeLa cells were grown in monolayer cultures at 34°C, and CHO cells were grown in suspension cultures at 34°C in a medium (25) containing deoxyribonucleotides (10 mg/ml) and 10% calf serum.

TS^+ transformations. Monolayer cultures of 4×10^5 to 1×10^6 cells on 100-mm tissue culture dishes were grown for 24 h at 34°C and then treated with 20 μg of DNA in 1.0 ml in the form of a calcium phosphate precipitate prepared exactly as described by Wigler et al. (27). After 24 h of exposure of the cells to DNA at 34°C, the medium was replaced and the cells were incubated at 40°C. Surviving TS^+ colonies were picked or scored after 2 to 3 weeks of growth at 40°C. All DNAs used for transformations were prepared as described by Wigler et al. (28).

RNA polymerase characterization. RNA polymerase II activities present in various cell lines were assayed (12) in aliquots of a cell lysate prepared as described previously (22). An activity resistant to inhibition by 30 μg of α-amanitin per ml, due to polymerases I and III and amounting to about 20% of the total activity, was subtracted in determining RNA polymerase II activity.

RESULTS

Transformation of TsAF8 cells with TS^+ BHK DNA. TsAF8, the TS mutant of the Syrian hamster BHK-21 cell line, may contain a TS RNA polymerase II mutation (22). Since TsAF8 cells will not grow at the nonpermissive temperature of 40°C, they can be used as recipients for transfer of the dominant TS^+ gene. A calcium phosphate-DNA precipitate of wild-type (TS^+) BHK-21 DNA was added to monolayer cultures of TsAF8 growing at 34°C by the modification of the transformation conditions of Graham and van der Eb (5) described by Wigler et al. (27). After 24 h of exposure to the DNA at 34°C, the medium was replaced and cells were exposed to the selection pressure, incubation at 40°C.

Surviving colonies were scored after 2 to 3 weeks of incubation at 40°C. The results of two representative experiments are shown in Table 1. The data for transformation of TsAF8 with TS^+ BHK-21 DNA indicated that the apparent frequency for TS^+ transformation on DNA-treated plates was only about threefold higher than the frequency of spontaneous reversion to the TS^+ phenotype on control plates that had not been exposed to DNA.

Normally, such a high and, as will be seen, somewhat variable frequency of reversion would preclude satisfactory demonstration of successful DNA-mediated transformation. However, previous studies from our laboratory suggested that the TS lesion in TsAF8 cells was a polymerase II mutation. This TsAF8 mutation was in the same complementation group as the RNA polymerase II mutations in CHO cells which lead to Ama^R and TS phenotypes (22). Ama^R mutations behave codominantly; that is, both wild-type and mutant genes are expressed

TABLE 1. TS^+ transformation of TsAF8 cell lines

Expt	Recipient cells	DNA	TS^+ colonies	
			No./ total plates	Frequency[a]
1	TsAF8	BHK-21	93/16	3×10^{-6}
		None	25/12	1×10^{-6}
2	TsAF8	BHK-21	57/12	2×10^{-6}
		None	13/11	6×10^{-7}
3	TsAF8-Ama^R14	BHK-21	7/5	4×10^{-7}
		None	0/3	$<2 \times 10^{-7}$
4	TsAF8-Ama^R14	BHK-21	3/9	3×10^{-7}
		None	1/8	6×10^{-8}
5	TsAF8-Ama^R14	Wild-type CHO	9/14	6×10^{-7}
		None	1/3	3×10^{-7}
6	TsAF8	Ama1	6/10	6×10^{-7}
		None	0/4	$<3 \times 10^{-7}$
7	TsAF8	Ama1	8/10	8×10^{-7}
		None	10/10	1×10^{-6}

[a] The frequency of TS^+ colonies is expressed as the number of TS^+ colonies obtained per cell exposed to DNA.

in heterozygous cells, and these cells grow in the presence of α-amanitin. Rather than use the α-amanitin-sensitive (Ama^s) TsAF8 cells as recipients for TS^+ (and therefore Ama^s) transformations, we used the TsAF8-Ama^R14 cell line, a derivative of TsAF8 in which we had introduced α-amanitin resistance as a second mutation in this TS complementation group (22). This allowed us to distinguish between spontaneous TS^+ revertants, now expected to be TS^+ but still Ama^R, and DNA-mediated TS^+ transformants, with a TS^+ Ama^s/TS Ama^R genotype and therefore Ama^R at 34°C but Ama^s at 40°C, the Ama^R allele of the recipient cell behaving in a temperature-sensitive fashion and therefore not conferring α-amanitin resistence to the transformed colonies growing at 40°C.

As indicated in Table 1, this Ama^R derivative of TsAF8 was also a suitable recipient cell line for DNA-mediated transformation. Although the apparent frequency of TS^+ transformation of TsAF8-Ama^R14 was somewhat lower than that for the parental TsAF8 cells, the spontaneous reversion rate was also reduced.

To distinguish between reversion and transformation, five TS^+ colonies from separate DNA-treated plates in these experiments were picked and grown up into mass cultures under selective conditions (40°C). The behavior of the α-amanitin resistance of each of these putative TS^+ transformants could now be examined. The recipient TsAF8-Ama^R14 cells and each of the putative transformants TR-1 through TR-5 were Ama^R at 34°C, plating at efficiencies between 8 and 90% in α-amanitin at 1.0 μg/ml (Table 2). Under the same conditions, the parental Ama^s cell line, TsAF8, was effectively killed. Of these five presumptive TS^+ transformants, only TR-1 was Ama^R at 40°C, whereas the remaining four cell lines, TR-2, TR-3, TR-4 and TR-5, were all killed at 40°C in the presence of α-amanitin. That is, TR-1, although picked from a DNA-treated plate, behaved as a TS^+ Ama^R spontaneous revertant, whereas the remaining colonies each behaved like the expected TS^+ Ama^s/TS Ama^R heterozygotes: Ama^R at 34°C and Ama^s at 40°C.

RNA polymerase II activities in TS^+ transformants. To provide more stringent evidence on the nature of the RNA polymerases in TS^+ transformed cell lines, we next examined the polymerase II activities in several of these cell lines. The mutant TsAF8-Ama^R14 did contain an altered, more α-amanitin-resistant polymerase II activity (22) (Fig. 1). Extracts of these cells were inhibited by concentrations of α-amanitin about threefold higher than the concentrations required to inhibit the parental Ama^s TsAF8 or wild-type BHK-21 polymerase II. The TS^+ transformant TR-5 (Fig. 1A) when grown at 40°C contained an Ama^s RNA polymerase II activity exactly like that of TsAF8 or BHK-21 cells. That is, at 40°C it expressed only the Ama^s allele characteristic of the donor Ama^s BHK-21 DNA. After short periods of growth at 34°C, these same cells contained a somewhat more resistant polymerase II activity; its titration curve showed the apparent coexpression at permissive temperatures of both the recipient cell Ama^r and the transferred Ama^s alleles. In contrast, the revertant TR-1 polymerase II was equally α-amanitin resistant at 34 and 40°C; its inactivation by α-amanitin was identical to that for the Ama^R polymerase II activity of TsAF8-Ama^R14 (Fig. 1B).

The results of these experiments demonstrated that the colonies selected at 40°C fell into two categories: transformants that expressed an Ama^s RNA polymerase II activity at 40°C characteristic of the donor DNA cell line and revertants that have Ama^R polymerase II activity at

TABLE 2. Effects of temperature and α-amanitin on growth of TS^+ isolates after BHK-21 DNA transformation of TsAF8-Ama^R14 cells

Cell line	Relative plating efficiency (%)[a] at:			
	34°C		40°C	
	Without α-amanitin	With α-amanitin	Without α-amanitin	With α-amanitin
TsAF8-Ama^R14	100	89	<0.2	<0.2
TsAF8	100	<0.2	<0.2	<0.2
TR-1	100	90	66	59
TR-2	100	8	85	<0.2
TR-3	100	37	91	<0.2
TR-4	100	26	89	<0.2
TR-5	100	54	69	<0.2

[a] Surviving colonies were stained and counted after 14 days of growth in the presence or absence of 1.0 μg of α-amanitin per ml. Values represent the number of colonies expressed as a percentage of number of colonies formed at 34°C in the absence of α-amanitin.

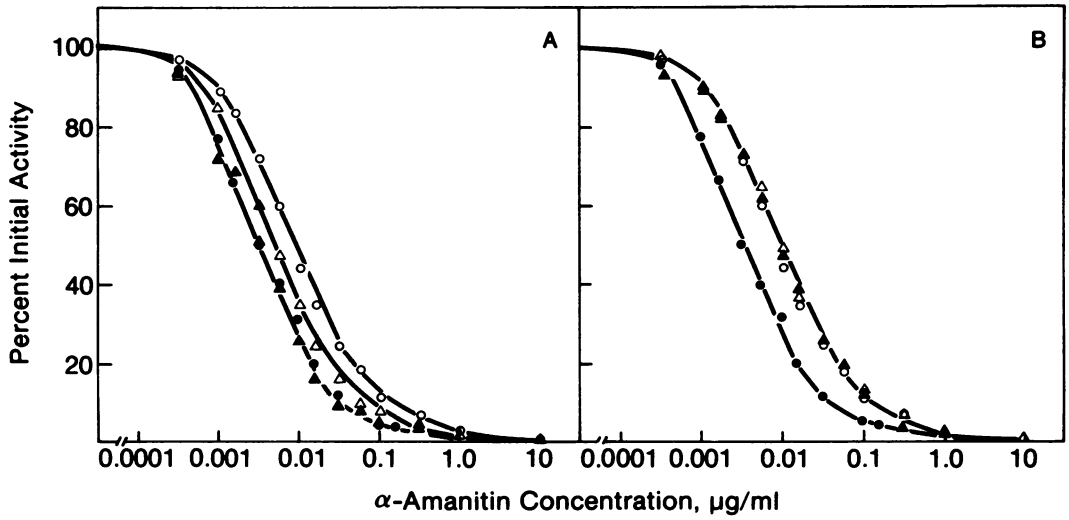

FIG. 1. Inhibition of TS and TS$^+$ cell RNA polymerase II activities by α-amanitin. RNA polymerase II activities present in cell lysates of Ama^s (TsAF8) and Ama^R (TsAF8-Ama^{R}14) cell lines and individual TS$^+$ isolates grown up in mass culture at 40°C and then at 40 or 34°C were assayed for 15 min at 30°C in the presence of increasing concentrations of α-amanitin: (A) TsAF8 (●), TS$^+$ TR-5 grown at 40°C (▲), TS$^+$TR-5 grown at 34°C (△), and TsAF8-Ama^{R}14 (○); (B) TsAF8 (●), TS$^+$ TR-1 grown at 40°C (▲), TS$^+$TR-1 grown at 34°C (△), and TsAF8-Ama^{R}14 (○).

40°C. The transformants not unexpectedly showed a variation in stability of the transferred TS$^+$ phenotype when maintained in culture for periods of time nonselectively at 34°C. TR-2 after approximately 30 generations of growth at 34°C replated at 40°C with an efficiency of only 3% relative to cells maintained at 40°C, whereas TR-3, TR-4, and TR-5 all appeared to be relatively stable transformants, replating at 40°C with relative efficiencies which ranged from 43 to 80%. The revertant TR-1 appeared completely stable and still plated at 40°C just as well as at 34°C after 30 generations of growth at 34°C.

TS$^+$ transformation with CHO DNA. Our initial transformations of the Syrian hamster cell line TsAF8 used homologous BHK-21 DNA as donor DNA. Although these experiments appeared to distinguish between spontaneous reversion and DNA-mediated transformation events, they did not exclude the possibility that reversions to TS$^+$ could involve reactivation of a silent Ama^s polymerase II gene in the recipient cell line. This mechanism of reversion, like transformation, would also generate the TS$^+$ Ama^s/TS Ama^R genotype observed in TS$^+$ cell lines like TR-5. To rule out this possibility, we performed DNA transformations with DNA from CHO cell lines in which well-characterized mutant alleles for α-amanitin-resistant RNA polymerase II exist (12).

DNA from wild-type CHO cells was as effective as BHK-21 DNA in transferring the TS$^+$ phenotype to the TsAF8-Ama^{R}14 cell line (Table 1). Four such TS$^+$ colonies were examined, and each of them was shown to be Ama^s in plating experiments at 40°C, yet still Ama^R at 34°C. Thus, there appeared to be no species barrier to transfer of an RNA polymerase gene between these two different hamster cell lines. We therefore expected that mutant alleles for the CHO RNA polymerase II could be transferred.

As shown in Table 1, treatment of the Ama^s cell line TsAF8 with DNA from the CHO mutant Ama1 also resulted in TS$^+$ colonies. However, as noted above (see Table 1), the spontaneous reversion of the TS mutation in TsAF8 is a frequent event which could obscure successful transformation. In fact in one experiment, for which data are shown in Table 1 (experiment 7), more colonies appeared on control plates than on the DNA-treated plates. Nevertheless, TS$^+$ colonies from both experiments were picked, grown up, and tested for the presence of a TS$^+$ Ama^R phenotype transferred from Ama1 cells. Not unexpectedly, a mixture of TS$^+$ transformants and spontaneous revertants was found. The growth of all three colonies from the DNA-treated plates of experiment 6 was entirely resistant to 2.5 μg of α-amanitin per ml. In experiment 7, one colony of three picked from plates that received Ama1 DNA was Ama^R, whereas all four colonies from control plates were Ama^s. In a similar set of experiments we also used DNA from the Ama^R cell line TR-1 described above in

transformation of TsAF8 cells and similarly obtained TS^+ Ama^R survivors at 40°C (data not shown).

We next examined the RNA polymerase II activities of a number of these TS^+ transformants that were shown to have acquired an Ama^R phenotype. In particular, we wished to see if the level of α-amanitin resistance of the polymerase II was that characteristic of the particular mutant cell line used as a source of DNA for the transformation. The Amal mutation renders the RNA polymerase II of Amal cells about 500-fold more resistant to inhibition by α-amanitin than wild-type Ama^s polymerase II (12). The RNA polymerase II activities in cell lysates from the TS^+ Ama^R transformants TrAmal-A and TrAmal-C were each examined for sensitivity to α-amanitin. When grown at 40°C, each of these cell lines had a polymerase II with a nearly monophasic inhibition curve, requiring a concentrations of α-amanitin similar to that required to inhibit the resistant polymerase II of the CHO Amal cells (Fig. 2A). On close examination, these curves revealed the presence of two distinctly different RNA polymerase II activities. When grown at 40°C, about 10 to 15% of their activities had a sensitivity to α-amanitin inhibition like that of the Ama^s TsAF8 polymerase II, whereas the remaining activity was like that of Amal cells, about 500-fold more resistant to α-amanitin inhibition. In contrast, these same cells when grown for 4 to 8 days at the permissive temperature of 34°C had RNA polymerase II activities whose titrations were clearly biphasic (Fig. 2B). Both TrAmal-A and TrAmal-C had approximately 50% of their polymerase II with a sensitivity to amanitin like that of Ama^s TsAF8 cells and 50% of their activity only inhibited by the concentrations of α-amanitin that inhibited the Amal polymerase II. In each case, the appearance of an increased proportion of Ama^s polymerase II activity after growth at 34°C for short periods was not due to any marked instability of the TS^+ Ama^R phenotype in the absence of selection at 40°C, for all of these TS^+ Ama^R transformants appeared to be reasonably stable over these same 4- to 8-day periods of growth at 34°C (plating data not shown). In analogous experiments in which DNA from the Ama^R cell line TR-1 described above was used to transform TsAF8 cells, an RNA polymerase II activity with a sensitivity to inhibition exactly like that in the TR-1 cell line (Fig. 1B) was seen in the putative TS^+ transformed cell line grown at 40°C. These results with transfer of the Ama^R phenotypes provided much more rigorous evidence that the TS^+ colonies can arise due to DNA-mediated transformation. It is quite clear that RNA polymerase II activity in these TS^+ transformants of TsAF8 cells was due to the expression of donor DNA sequences in this TS cell line rather than to silent TS^+ gene reactivation.

TS^+ transformation with human DNA. Since

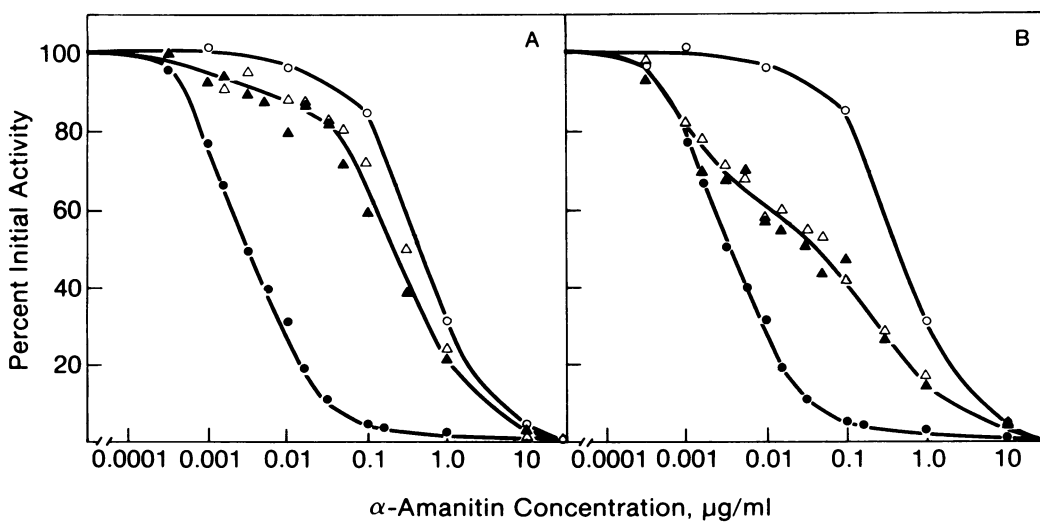

FIG. 2. Inhibition of TS and TS^+ transformed cell RNA polymerase II activities by α-amanitin. RNA polymerase II activities present in cell lysates of the recipient TsAF8 and DNA donor CHO Amal cell lines and two independent TS^+ transformants, TrAmal-A and TrAmal-C, were assayed for 15 min at 30°C in the presence of increasing concentrations of α-amanitin: (A) TsAF8 (●), CHO Amal (○), TrAmal-A grown at 40°C (▲), and TrAmal-C grown at 40°C (△); (B) TsAF8 (●), CHO Amal (○), TrAmal-A grown at 34°C (▲), and TrAmal-C grown at 34°C (△).

the CHO DNA proved suitable for TS^+ transformation of the Syrian hamster cell line, it was of interest to see if more distantly related mammalian DNAs were also capable of this TS^+ transformation. We used human DNA prepared from both HeLa cells and an Ama^R human diploid fibroblast strain, Ama1070, described earlier by Buchwald and Ingles (2). These human DNAs were indeed capable of transformation of the TsAF8 cell line. In one experiment the frequency of TS^+ transformation with HeLa cell DNA was 2.2×10^{-5}, higher than that observed with any hamster DNA; on untreated control plates the spontaneous TS^+ reversion frequency at that time was 1.2×10^{-6}. Two independant TS^+ isolates obtained after transformation of TsAF8 cells with the Ama1070 human fibroblast DNA were shown to be Ama^R in plating experiments. They contained an RNA polymerase II activity when grown at 40°C that was similar in its level of resistance to the resistant activity in the Ama1070 fibroblasts (data not shown).

DISCUSSION

In these studies we have shown that the gene for mammalian RNA polymerase II which determines sensitivity to inhibition by α-amanitin can be transferred to a TS Syrian hamster BHK-21 cell line by selection for survival at the nonpermissive temperature. DNA-mediated gene transfer of the codominantly expressed Ama^R phenotype was an obvious candidate for such gene transfer experiments. However, earlier experiments in this and other laboratories in which growth in the presence of α-amanitin was used to select Ama^R transformants after treatment of both CHO and mouse Ltk^- cell lines with DNA from Ama^R CHO mutants were never fruitful. The CHO cell lines are notoriously poor recipients for DNA transformation, but it is not immediately apparent why transfer to the quite competent Ltk^- cell line was unsuccessful. One explanation may be that α-amanitin too rapidly inactivates RNA polymerase II activity, whereas the half-life of polymerase II of the TS mutant TsAF8 when shifted to 40°C is at least 12 to 15 h and the cells remain viable at nonpermissive temperatures for as long as 40 h (20, 21). Retention of some recipient cell RNA polymerase II activity during the early stages of selection of transformants may be essential.

The TsAF8 cell line is a TS mutant of BHK-21 cells selected by 5-fluoro-2'-deoxyuridine killing of dividing cells at nonpermissive temperatures (17). It arrests at nonpermissive temperatures in the mid-G_1 phase of the cell cycle (3). Previous studies of this mutant suggested that its RNA polymerase II could be the function which has the TS mutation. Growth at 40°C results in a selective loss of the activity of RNA polymerase II (20) and a loss of polymerase II molecules as quantitated by [^{3}H]amanitin binding (21). More importantly, this TS mutation in TsAF8 was complemented by fusion with wild-type CHO cells, but not by fusion with the CHO cell TS polymerase II mutant $TsAma^R$-1 (22). We have interpreted these data as implying that the TS Ama^R mutations (11) of CHO cell lines and the TS mutation in TsAF8 cells are mutations in the same complementation group. The gene is likely to be the gene coding for the 140,000-dalton subunit of polymerase II, the subunit to which amanitin derivatives have been covalently cross-linked (1).

To demonstrate that the TS^+ transformation events were indeed DNA mediated, it was necessary to clearly distinguish between spontaneous TS^+ reversion of TsAF8 and the true TS^+ transformation. As our data have indicated, spontaneous reversion of the TS mutation in TsAF8 cells can be a serious problem. If the Ama^R mutation and the TsAF8 mutation are in the same polypeptide of polymerase II, then we expected to see a new Ama^s polymerase II activity when TS^+ Ama^s DNA transformed the TsAF8-Ama^R14 cell line and a new Ama^R polymerase II activity when TS^+ Ama^R DNA was used to transform (Ama^s) TsAF8 cells. In fact this was observed. In our experiments, the polymerase II activity present in putative TS^+ transformants expressed the phenotype at 40°C expected of the transforming DNA; that is, Ama^s with BHK-21, wild-type CHO, and HeLa DNAs and Ama^R in the case of CHO Ama1, TsAF8-TR-1, and Ama1070 DNAs. Such Ama^R or Ama^s phenotypes were demonstrated by plating experiments. Acquisition of the appropriate polymerase II activity was more rigorously shown by titrations of the RNA polymerase II activities by α-amanitin in each of the TS^+ transformed cell lines—an assay that clearly distinguishes between different Ama^R polymerase II mutations. The appearance of new forms of polymerase II in many of the TS^+-selected colonies identified the bona fide transformed cells and supported the suggestion that the TS and Ama^R mutations are in the same gene. Since such a DNA transformation was also consistent with the TS^+ and Ama^R mutations being only physically linked or otherwise cotransferred genes, it is important to note that after transfer of TS^+ Ama^s DNA to the TS Ama^R cell line TsAF8-Ama^R14, expression of Ama^R polymerase II activity was only seen at the permissive temperature of 34°C. Were the TS^+ and Ama^R genes coding for different polypeptides, a TS^+ Ama^R activity should also have been expressed and transformed colonies should have been Ama^R at 40°C.

Since the recipient cell polymerase II activity is TS, the TS^+ transformants at 40°C contained

largely donor-DNA-derived RNA polymerase II activity. Close examination of the titration by α-amanitin of the polymerase II activity of TS^+ transformants TrAma1-A and TrAma1-C indicated that a minor proportion of Ama^s polymerase II activity was still detectable (Fig. 2A). This is not unreasonable, given the relatively slow disappearance of TsAF8 polymerase II activity upon shift of TsAF8 cells to nonpermissive temperatures (20, 21). It is likely that in the presence of a TS^+ activity there is continued synthesis of the TsAF8 polymerase II and relatively slow inactivation by temperature of this TS activity. The presence of both Ama^s and Ama^R polymerase II activities in these same TS^+ transformants was strikingly demonstrated in the biphasic inhibition curves of the activities from cells grown at 34°C (Fig. 2B). It appears that the transferred Ama^R gene is expressed exactly in the same 1:1 proportion relative to the recipient cell Ama^s polymerase II gene as when Ama^R/Ama^s hybrid cell lines were constructed by cell fusion (7). Expression of DNA transferred genes for the cellular enzymes thymidine kinase (15) and adenine phosphoribosyltransferase (27), enzymes whose expression may normally be constitutive, was similar to expression in wild-type murine fibroblasts. In contrast, transfer to the Ltk^- murine fibroblasts of such genes as those for globin and ovalbumin, whose expression is usually restricted to differentiated cell types, overcame the negative regulation controlling their expression (14, 16). The Ama^R polymerase II gene in these transformed cell lines, like the rat α_{2u} globulin (13) and mouse mammary tumor virus proviral (9) genes, has clearly been transferred in such a manner that elements which serve in the regulation of its expression are still intact.

We have shown in this study that transfer of a gene for Syrian or Chinese hamster polymerase II and also human polymerase II is effective in complementing the TS defect in the Syrian hamster TsAF8 cells. We cannot exclude the possibility that all of the 7 to 14 subunit polypeptides of polymerase II are genetically very closely linked and capable of being cotransferred on a single DNA molecule. It seems more likely, however, that in each case the transfer involves only the DNA coding for a single subunit of polymerase II and that a hybrid CHO-BHK hamster or human-hamster enzyme results. This hybrid enzyme is capable of effective gene transcription. This implies that mammalian RNA polymerase II structure is extremely well conserved—a finding in accordance with both the antigenic homologies detected between polymerases II from different species (10) and the similarities in the subunit polypeptide sizes of many of the mammalian polymerase II enzymes. Furthermore, it suggests that current experiments measuring in vitro transcription of cloned eucaryotic genes with mammalian polymerase II purified from a different species (26) may not obscure aspects of transcriptional control.

The successful DNA-mediated transfer of this RNA polymerase II gene is the crucial step in a viable approach to obtaining recombinant clones of this gene. Such recombinant clones will facilitate analysis of regulatory mechanisms and mutations affecting RNA polymerase II in cultured cell lines. Perhaps even more importantly, they may lead to an understanding of the abnormalities in specific development pathways caused by mutation (6, 18) of this same locus in other organisms, such as *Drosophila melanogaster*.

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