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In Vivo Regulation of rRNA Transcription Occurs Rapidly in Nondividing and Dividing *Drosophila* Cells in Response to a Phorbol Ester and Serum

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The synthesis of ribosomes is an essential cellular process which requires the transcription of the rRNA genes by RNA polymerase I (Pol I). The regulation of rRNA synthesis is known to be coupled to growth regulation. In nongrowing, slowly growing, and rapidly growing *Drosophila* cells, exposure to the tumor-promoting phorbol ester 12-*O*-tetradecanoylphorbol-13-acetate (TPA) increases the synthesis of precursor and mature rRNAs. Using nuclear run-on assays, we show that TPA enhances transcription of the rRNA genes. These results suggest that TPA regulates expression of rRNA genes transcribed by Pol I, irrespective of the growth state of the cells. In slowly dividing *Drosophila* cells, increasing the serum concentration rapidly alters the accumulation of rRNA by enhancing rDNA transcription within 1 h. Thus, TPA and serum are each able to rapidly regulate rRNA gene expression in *Drosophila* cells. These results indicate that the RNA Pol I transcription system can be regulated by agents which have previously been shown to effect specific genes transcribed by the RNA Pol II system.

It is now widely accepted that tumor-promoting phorbol esters, which mimic the action of diacylglycerol, can activate a calcium- and phospholipid-dependent protein kinase, protein kinase C (reviewed in references 4, 17, and 20). We have previously observed that protein synthesis can be stimulated within minutes in nondividing *Drosophila* cells by reagents that act through a pathway involving calcium and the activation of protein kinase C (32). We found that phorbol esters stimulated protein synthesis and that this stimulation was dependent on the presence of calcium, suggesting a common signaling pathway. Since in most systems, the cellular processes of protein and rRNA synthesis are closely coupled (reviewed in reference 21), we have investigated whether in nondividing cells, activation of protein kinase C by 12-*O*-tetradecanoylphorbol-13-acetate (TPA) can bring about changes in rRNA synthesis parallel to its affects on protein synthesis.

Since TPA is known to affect growth-related events in dividing cells, we also chose to investigate the effects of this reagent on rRNA synthesis in dividing *Drosophila* cultured cells which were either growing slowly, i.e., under serum-reduced conditions, or growing rapidly, in the presence of a high concentration of serum.

There are several levels at which stimulation or inhibition of RNA synthesis could potentially be controlled. We have investigated the changes in rDNA transcription which take place in *Drosophila* cells treated with TPA and serum by using nuclear run-on assays to examine the number of active polymerase I (Pol I) complexes present on the 18S and 28S rRNA genes. Certain genes transcribed by RNA Pol II are known to be induced by phorbol ester and serum (reviewed in reference 12). Here, we demonstrate that the transcription of rRNA genes by RNA Pol I is rapidly increased by a phorbol ester and serum.

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MATERIALS AND METHODS

***Drosophila* culture and male accessory gland isolation.** Wild-type *Drosophila melanogaster* (Oregon-R) was grown at 25°C on a standard cornmeal-Karo-yeast agar medium supplemented with live yeast cells. After eclosion, the flies were separated according to sex. Accessory glands from 10 virgin males (7 to 10 days old) were dissected free of testicular and gut tissue and placed at 4°C in morpholinepropanesulfonic acid (MOPS) buffer (10 mM MOPS [pH 7.0], 80 mM NaCl, 10 mM KCl, 0.2 mM $MgCl_2$, 0.1 mM $CaCl_2$).

rRNA labeling in male accessory glands. Dissected glands were incubated at room temperature for 15 min in 50 μl of MOPS buffer with 50 nM TPA. The medium was removed; the glands were washed once and then resuspended in MOPS buffer. Twenty-five microcuries of $^{32}P_i$ (9,000 Ci/mmol; Amersham) was added, and incubation was continued for 1 h at room temperature. The control glands were treated in a similar fashion, using MOPS buffer for all procedures.

Maintenance of cell cultures. Schneider Line-2 tissue culture cells were maintained in *Drosophila* Schneider's medium (GIBCO) supplemented with 0.1% Yeastolate (Difco), 0.25% Bacto Peptone (Difco), 1% antibiotic-antimycotic (Sigma), and 2 or 15% fetal bovine serum (FBS; GIBCO). Cells were adapted to 2% FBS by gradually reducing the serum content in the medium from 15 to 2% over a period of 3 weeks. Gradual reduction in serum content did not result in significant cell death as determined, by trypan blue staining. Cells growing in 2% FBS doubled every 50 h, whereas cells growing in 15% FBS doubled every 24 h.

In vivo labeling of rRNA in tissue culture cells. Cells from mid-log-phase cultures were harvested, washed, and counted. The cells were resuspended in the appropriate medium and aliquoted at 1×10^7 to 2×10^7 cells per ml. Following equilibration (30 to 60 min, room temperature), the cells were incubated with various concentrations of phorbol esters (0.5 to 500 nM) for 15 min. The cells were pelleted and then resuspended in fresh medium containing 100 μCi of $^{32}P_i$ (9,000 Ci/mmol; Amersham) per ml and

labeled for 1 h at room temperature. In the case of serum treatment, cell pellets were resuspended in medium containing 15% FBS for 1, 2, or 3 h and labeled only in the last hour of the treatment period.

RNA isolation and analysis. Total RNA was isolated from the male accessory glands by using the hot phenol method of Pelham (22). Total RNA was isolated from tissue culture cells by using the LiCl method of Cathala et al. (7). The RNA was glyoxylated as described by McMaster and Carmichael (19) and electrophoresed on 1.5% agarose gels (15). The gels were stained with ethidium bromide (0.5 μg/ml) and photographed so that the RNA recovered and loaded on each lane could be quantitated by densitometry. The gels were then dried, and the labeled RNA was visualized by autoradiography. Multiple autoradiograms were scanned with an LKB densitometer, and integrated areas corresponding to rRNA species were summed. All results presented are averages of at least two independent experiments.

Nuclear isolation from dissected male accessory glands. Glands were dissected from 7- to 10-day-old virgin male flies and stored on ice in 1 ml of MOPS buffer until 200 glands were collected. Glands were brought to 25°C before exposure to reagents, after which they were washed in MOPS buffer. Glands were resuspended in 1 ml of MOPS buffer containing collagenase (1 mg/ml; Cooper Biochemical) and then incubated at 37°C. Glands were vortexed for 5 s once every 5 min until intact tissue was no longer visible and the solution was cloudy. The reaction mixture was centrifuged at 14,000 × *g* for 10 min at 4°C, and the nuclei were then treated with RNase A (100 μg/ml, 30 min, 4°C) and purified over sucrose pads as described by Schibler et al. (23). Nuclei from glands were resuspended in nuclei suspension buffer (20 mM Tris HCl [pH 7.9], 75 mM NaCl, 0.5 mM EDTA, 0.85 mM dithiothreitol, 0.125 mM phenylmethylsulfonyl fluoride, 5 mM $MgCl_2$, 50% glycerol) and used immediately in transcription reactions.

Isolation of nuclei from Schneider Line-2 Cells. All procedures for the isolation of nuclei were carried out at 4°C. Cells were incubated with the various reagents as described for the in vivo labeling experiments. Pelleted cell suspensions were resuspended in 0.3 M sucrose in lysis buffer A (60 mM KCl, 15 mM NaCl, 0.15 mM spermine, 0.5 mM spermidine, 14 mM 2-mercaptoethanol, 0.5 mM EGTA, 2 mM EDTA, 15 mM *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid [HEPES; pH 7.0]), lysed with 0.2% Nonidet P-40 for 5 min on ice, and then centrifuged (2,500 × *g*, 30 min, 4°C) over 20% sucrose pads in lysis buffer A (19). The nuclei were then treated with RNase A (100 μg/ml) in lysis buffer A for 30 min at 4°C. RNase A-treated nuclei were further purified by two successive centrifugations (2,500 × *g*, 30 min, 4°C) over 20% sucrose pads in lysis buffer A. The nuclei were resuspended in 0.3 M sucrose in lysis buffer B (same as buffer A, but containing 0.1 mM EGTA and 0.1 mM EDTA) and purified by ultracentrifugation (150,000 × *g*, 60 min, 4°C) over 2 M sucrose pads in lysis buffer B. Nuclei were then resuspended at 10^6 nuclei per μl in nuclei suspension buffer and stored at −70°C. Aliquots were removed for quantitation by hemocytometer counting, using 0.4% crystal violet in 0.1 M citric acid, which gives an accurate measure of intact nuclei.

Nuclear run-on transcription assays. RNase A-treated nuclei (5×10^7) were incubated in the presence of α-amanitin (100 μg/ml; Sigma) for 5 min on ice. Samples were transferred to 26°C, and the transcription reactions were continued for 10 min in a buffer containing 60 mM Tris HCl (pH 7.9), 62.5 mM NaCl, 0.45 mM EDTA, 0.625 mM dithiothreitol, 0.112 mM phenylmethylsulfonyl fluoride, 350 mM $(NH4)_2SO_4$, 1 mg of heparin sulfate per ml, 4 mM $MnCl_2$, 5 mM $MgCl_2$, 0.625 μM [α-^{32}P]UTP (800 Ci/mmol; NEN), 1 mM each ATP, CTP, and GTP (Pharmacia), 10 mM creatine phosphate, 130 U of RNasin (Promega) per ml, and 30% glycerol. The reactions were terminated by lysis in 10 volumes of 4 M guanidinium thiocyanate–25 mM sodium citrate (pH 7.0)–0.5% Sarkosyl–0.1 mM 2-mercaptoethanol, and labeled RNA was extracted by the single-step method of Chomczynski and Sacchi (9). Fifty-six percent of the total nuclear transcriptional activity was determined to be α-amanitin insensitive. In the presence of α-amanitin, transcription reactions typically incorporated 5.1×10^5 to 9.2×10^5 dpm (0.23 to 0.42%). α-Amanitin-insensitive transcripts synthesized after incubation for 10 min at 26°C ranged in size from 1,400 to 1,000 nucleotides, from which an in vitro elongation rate of 2.0 nucleotides per s is estimated. This rate is in agreement with that reported by others (18, 23).

Run-on transcripts were hybridized in buffer containing 50% formamide, 0.1% sodium dodecyl sulfate (SDS), 1× Denhardt's reagent, 5× SSC (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate), of salmon sperm DNA per ml, and 20 mM sodium phosphate (pH 6.5) to Southern or slot blots (16) of pAB1915 and pAB2103. pAB1915 contains a 1.03-kb *Bgl*II-*Eco*RI fragment of 18S rDNA cloned in the *Eco*RI site of pBR322. pAB2103 contains a 0.97-kb *Hin*dIII-*Hin*dIII fragment of 28S beta sequence cloned in the *Hin*dIII site of pBR325 (Fig. 1). pAB clones were derived from a 3S18-containing clone (2). In every case, rDNA sequences were present in equimolar amounts. The amount of membrane-bound rDNA was determined to be in excess over complementary rRNA by hybridizing ^{32}P-labeled run-on transcripts to separate filters containing increasing amounts of each rDNA plasmid. DNA excess was defined as the concentration at which the hybridization signal was no longer proportional to the concentration of DNA present.

The membranes were washed at low stringency (2× SSC–0.1% SDS, 5 min, 25°C) and then washed twice at high stringency (0.2× SSC–0.1% SDS, 30 min, 55°C). Hybridized rRNA transcripts were visualized by autoradiography. Multiple exposures were obtained and scanned with an LKB scanning densitometer. Changes in transcription in response to treatment with each reagent were determined by calculating the change in hybridization signal of 18S and 28S sequences relative to the level of hybridization observed in untreated nuclei. Results presented represent averages of at least two independent experiments.

RESULTS

A tumor-promoting phorbol ester, TPA, stimulates rRNA synthesis in nondividing *Drosophila* cells. The male accessory glands are a terminally differentiated tissue which responds metabolically to calcium changes and activation of protein kinase C and therefore serves as a useful system in which to study the potential effects of TPA on rRNA gene expression. This secretory tissue produces proteins found in the seminal fluid which have important effects on female reproductive behavior (reviewed in reference 8). We previously established that the male accessory glands are a nondividing tissue, since no labeled nuclei are observed following incubation of the glands with [^{3}H]thymidine for 24 h (25). Although mating induces both protein and rRNA synthesis in the glands, it does not induce DNA synthesis or cell division (24). Furthermore, we developed an in vitro system for these *D. melanogaster* glands (32). Using our in vitro system, we observed that added calcium, activation of

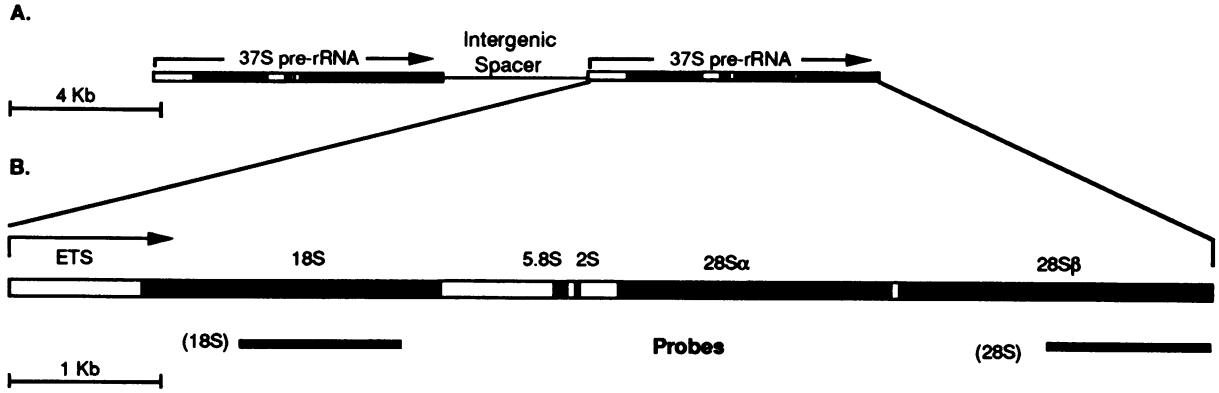

FIG. 1. Structure of a *Drosophila* rDNA repeat. (A) The multiple copies of rRNA genes, coding for the 37S RNA precursor to the 18S, 5.8S, 2S, and 28S rRNAs, are arranged in tandem arrays. Each gene region is separated from the next by an intergenic spacer. (B) The 7.3-kb *Drosophila* rDNA repeat encodes the 18S, 5.8S, 2S, and 28S rRNA genes. In *D. melanogaster*, the 28S rRNA is further processed into the 28S α and 28S β rRNAs, which remain base paired to each other in the ribosome. Transcription of the primary pre-rRNA transcript initiates at the gene promoter located in the external transcribed spacer (ETS). The DNA regions used as probes in nuclear run-on transcription experiments are provided for reference. Their designations are descriptive only and do not represent those rDNA regions in their entirety.

protein kinase C by TPA, and treatment with juvenile hormone each increased protein and total RNA synthesis, accurately reflecting the mating-induced stimulation of overall protein synthesis observed in vivo (32).

To study the effects of protein kinase C-mediated signals on rRNA synthesis in a terminally differentiated tissue, glands were incubated in vitro with various concentrations of phorbol esters, after which they were labeled for 1 h with $^{32}P_i$. Total RNA was then extracted, and the rRNA was analyzed by gel electrophoresis. It should be noted that in *D. melanogaster*, the 28S rRNA is rapidly processed into two species, 17S and 19S (14), which migrate near the 18S rRNA in our gel system.

When glands are incubated with 50 nM TPA for 15 min, rRNA labeling increased threefold (Fig. 2A). Exposure of the glands to the protein kinase C-inactive compound, phorbol, at the same concentration did not stimulate rRNA labeling; rather, phorbol inhibited synthesis (Fig. 2A). Thus, TPA stimulates the accumulation of rRNA in nondividing *Drosophila* cells.

Since we observed increased rRNA synthesis in the male accessory glands exposed to TPA, we wished to determine whether these changes reflected a change in the level of rDNA transcription. Nuclei were isolated and then incubated in the presence of [α-^{32}P]UTP for 10 min to allow engaged polymerases to elongate. Previous experiments indicated that there was linear incorporation of label into RNA for at least 30 min. These transcription reactions were done in the presence of high concentrations (100 μg/ml) of α-amanitin to ensure that we were measuring RNA Pol I activity. The inclusion of heparin in the run-on transcription assays prevented reinitiation by polymerases and inhibited nucleases (5, 11, 18).

The labeled run-on RNA was isolated and then hybridized to membrane-immobilized DNAs. Plasmid DNAs containing 1-kb fragments from the 3′ end of the 18S segment and the 3′ end of the 28S segment diagrammed in Fig. 1 were filter bound such that equimolar amounts of rDNA sequences were present in each slot. We determined that 18S and 28S rDNA sequences were present in excess over the complementary rRNA synthesized in vitro. We chose a probe specific for the 18S rDNA sequence rather than the external transcribed spacer to avoid assaying reinitiation events and to assay stable RNA, since the external transcribed spacer is rapidly degraded in vivo. To eliminate the possibility that we were observing nonspecific hybridization, vector DNA was also probed with run-on RNA. The 18S and 28S gene regions which we used to measure the loading of active transcription complexes are greater than 1.4 and 6.3 kb, respectively, from the start site of transcription (diagrammed in Fig. 1). Therefore, results from nuclear run-on transcription reactions reflect the relative distribution of active RNA Pol I transcription complexes on the 18S and 28S rDNA sequences after various experimental treatments. Differences in the amount of transcription detected from a given gene region could be the result of increased transcription initiation (polymerase loading) and/or elongation.

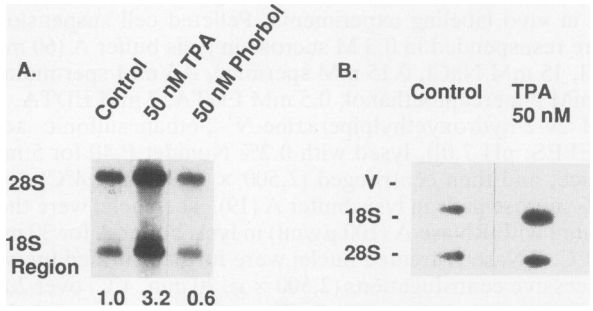

FIG. 2. Regulation by TPA of rRNA synthesis in nondividing male accessory gland cells. (A) Effects of phorbol esters on rRNA labeling in nondividing male accessory gland cells. Equal numbers of dissected male accessory glands were incubated in MOPS buffer with or without TPA and then labeled with 25 μCi of $^{32}P_i$ per ml for 1 h, after which total RNA was extracted and analyzed on 1.5% agarose gels. Shown are autoradiograms of rRNA from glands treated with 0.1 mM $CaCl_2$ (control) and glands treated with 0.1 mM $CaCl_2$ plus 50 nM TPA or phorbol. Numbers below the lanes correspond to relative changes in rRNA labeling as determined by densitometry. (B) RNA Pol I loading of rDNA sequences in control and phorbol ester-treated male accessory glands. Nuclei were isolated from 200 glands that had or had not been exposed to TPA and were transcribed in the presence of 100 μCi of [α-^{32}P]UTP and 100 μg of α-amanitin per ml for 10 min. Labeled transcripts were hybridized to membrane-bound 18S rDNA, 28S rDNA, or vector (V) sequences.

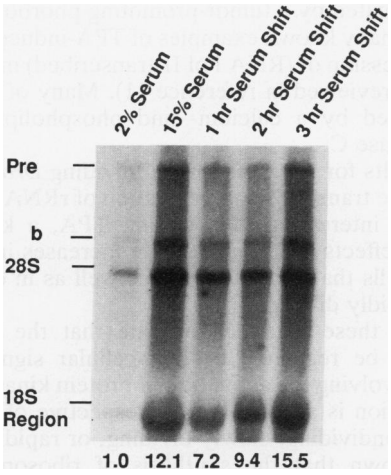

FIG. 3. In vivo labeling of rRNA in serum-shifted Schneider Line-2 cells. Equal numbers of cultured cells were incubated with serum for the indicated times and labeled with 100 μCi of $^{32}P_i$ per ml for 1 h, after which total cellular RNA was extracted and analyzed as described in the legend to Fig. 2. Cells treated with serum for 2 or 3 h were labeled only in the last hour of the treatment period. Precursor RNA (Pre) and processed intermediate (b) are indicated. Numbers below the lanes correspond to relative changes in rRNA labeling as determined by densitometry.

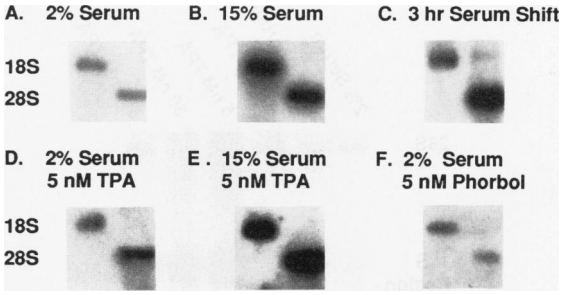

FIG. 4. RNA Pol I loading of rDNA sequences in serum- or phorbol ester-treated Schneider Line-2 cells. RNase-treated nuclei (5×10^7) from untreated, serum-stimulated, or TPA-treated cells were transcribed in the presence of 100 μg of α-amanitin per ml and 100 μCi of [α-^{32}P]UTP for 10 min. Radiolabeled transcripts were hybridized to membrane-immobilized sequences from the 18S and 28S regions. Shown are autoradiograms of labeled transcripts synthesized by the nuclei of cells grown in medium containing 2% serum (A), cells grown in medium containing 15% serum (B), cells grown in medium containing 2% serum and shifted to medium containing 15% serum for 3 h (C), cells grown in medium containing 2% serum and treated with TPA for 15 min (D), cells grown in medium containing 15% serum and treated with TPA for 15 min (E), and cells grown in 2% serum and treated with phorbol for 15 min (F).

Nuclei isolated from glands incubated in the absence of TPA for 1 h, with no other treatment, yielded labeled run-on RNA that hybridized to a similar extent to the 18S and 28S rDNAs (Fig. 2B). Similarly, when nuclei from glands were incubated in medium containing 50 nM TPA for 15 min, run-on RNA increased fourfold (Fig. 2B). Therefore, exposure of the nondividing *Drosophila* cells to TPA results in increased distribution of active RNA Pol I transcription complexes on the 18S and 28S rDNA sequences.

Synthesis of rRNA in dividing *Drosophila* cells is correlated with growth rate and serum exposure. Having observed the stimulation of rRNA synthesis in nondividing cells, we wished to examine the regulation of rRNA synthesis in dividing *Drosophila* cells. Schneider Line-2 cells can be grown either continuously in medium containing 15% serum (doubling time of 24 h) or in medium containing 2% serum (doubling time of 50 h). They are referred to here as rapidly or slowly growing cells, respectively.

When equal numbers of cells are labeled for 1 h with $^{32}P_i$, a 12-fold increase in rRNA labeling is observed in cells growing rapidly in 15% serum compared with cells growing slowly in 2% serum (Fig. 3). The lane corresponding to the rapidly growing cells was purposely overexposed so that rRNA labeling in the slowly growing cells could be visualized. These results are those expected from cells growing at a reduced rate (30).

A large and rapid increase in rRNA synthesis was observed when slowly growing cells were exposed to medium containing 15% serum. During the first hour of serum exposure, the amount of rRNA labeling increased sevenfold over that observed in untreated cells (Fig. 3). Exposure to serum for 2 h with labeling during the second hour increased rRNA synthesis ninefold (Fig. 3). Exposure for 3 h with labeling during the third hour increased rRNA synthesis 15-fold (Fig. 3). rRNA synthesis in serum-stimulated cells following 3 h of serum exposure often exceeded that observed in rapidly growing cells maintained continuously in 15% serum (Fig. 3).

Our labeling and isolation methods allow us to directly observe the precursor and several processed intermediates of the mature 18S and 28S rRNAs. We had previously determined preribosomal and processed rRNA intermediates in our gel system (3) which directly corresponded to those reported by Long and Dawid (14). As seen in Fig. 3, [^{32}P]phosphate label accumulates in both the precursor and processed intermediates upon serum exposure. In addition, processing of the pre-rRNA transcript appears to keep pace with the changes in rRNA labeling. Therefore, because of the proficiency of the processing reactions, it seems unlikely that processing regulates rRNA synthesis under the conditions that we examined.

We then investigated the changes in rDNA transcriptional activity in serum-stimulated *Drosophila* cells. Labeled run-on RNA was isolated and hybridized to Southern blots containing equimolar amounts of 18S and 28S rDNA sequences. In each case, plasmid DNA was in DNA excess over complementary rRNA. When an equal number of nuclei isolated from rapidly growing cells were assayed in nuclear run-on experiments, a threefold-higher level of transcripts homologous to 18S and 28S sequences was observed compared with that seen in nuclei from the more slowly growing cells (Fig. 4A and B). Within 3 h of exposure to 15% serum, slowly growing cells yield levels of run-on transcripts equal to those detected in cells maintained continuously in 15% serum (Fig. 4C).

These results indicate that rRNA synthesis in dividing *Drosophila* cells parallels the growth state of the cells. Increases in rRNA accumulation in response to serum stimulation of slowly growing cells are due to increased transcription of the rRNA genes. In contrast, the nondividing cells of the *Drosophila* male accessory gland are not stimulated by the addition of serum (32). Therefore, unique to the dividing *Drosophila* cells, regulation of rDNA transcription occurs in response to a signaling pathway involving serum factors.

TPA regulates rRNA synthesis in dividing *Drosophila* cells. We have examined the effects of protein kinase C-mediated

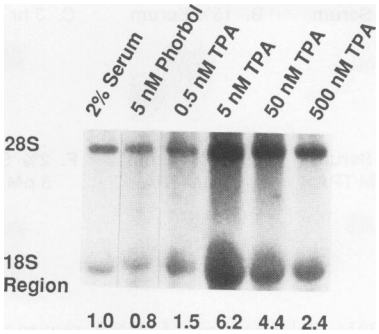

FIG. 5. Effects of phorbol esters on rRNA labeling in Schneider Line-2 cells. Equal numbers of cells were treated with various concentrations of phorbol esters for 15 min, washed, and resuspended in fresh medium prior to labeling with 100 μCi of $^{32}P_i$ per ml for 1 h. Total cellular RNA was extracted and analyzed on 1.5% agarose gels. Numbers below the lanes correspond to the relative changes in rRNA as determined by densitometry.

signals on the regulation of rRNA synthesis in dividing *Drosophila* cells as well. We wanted to determine whether there were any differences in the ability of phorbol esters to stimulate rRNA synthesis in slowly growing cells compared with those already growing rapidly.

Slowing growing cells treated with 500 nM TPA for 15 min showed a twofold increase in rRNA labeling, while treatment with 50 nM TPA increased rRNA labeling fourfold (Fig. 5). A maximal increase (6-fold) in rRNA labeling was seen when these cells were treated with 5 nM TPA, while with 0.5 nM TPA treatment, a 1.5-fold stimulation was observed (Fig. 5). Rapidly growing cells treated with 5 nM TPA show only a twofold increase in rRNA labeling (data not shown). Treatment of slowly growing cells with 5 nM phorbol, a non-tumor-promoting compound, did not increase rRNA accumulation compared with untreated cells; rather, it decreased labeling (Fig. 5).

We again investigated the transcriptional activity of both 18S and 28S rRNA gene sequences in these cells. Upon exposure of slowly growing cells to 5 nM TPA, a twofold-higher level of transcripts homologous to rDNA gene sequences was seen (Fig. 4D). Treatment of an equal number of rapidly growing cells with 5 nM TPA resulted in an additional increase in transcripts detected from the 18S and 28S rDNA regions (Fig. 4E). It is significant that rapidly growing cells could be stimulated a further twofold by exposure to TPA (compare Fig. 4B and E). As an additional control, we found that treatment of slowly growing cells with 5 nM phorbol did not significantly increase transcripts homologous to rRNA gene sequences (Fig. 4A and F).

Therefore, results for dividing *Drosophila* cells indicate that TPA, probably through activation of protein kinase C, regulates rDNA transcription. TPA can also regulate rRNA synthesis in the nondividing cells of the *Drosophila* male accessory gland. Therefore, regardless of the growth state of the cell, rapid regulation of rDNA transcription occurs in response to a signaling pathway involving the phorbol ester TPA.

DISCUSSION

The cellular signaling pathways involved in the regulation of eukaryotic ribosome synthesis are not well established. Results presented here demonstrate that in dividing and in nondividing *Drosophila* cells, regulation of rRNA synthesis can be mediated by a tumor-promoting phorbol ester, TPA. There are many known examples of TPA-induced alterations in the expression of (RNA Pol II-transcribed) mRNA encoding genes (reviewed in reference 11). Many of these effects are mediated by a calcium- and phospholipid-dependent protein kinase C.

Our results for nondividing and dividing *Drosophila* cells demonstrate transcriptional regulation of rRNA synthesis by TPA. It is interesting to note that TPA, a known tumor promoter, effects the same relative increases in rRNA synthesis in cells that cannot divide as well as in cells that are already rapidly dividing.

In total, these data demonstrate that the synthesis of rRNA can be regulated by intracellular signaling events possibly involving the activation of protein kinase C and that this regulation is accomplished irrespective of whether the cells are nondividing, slowly dividing, or rapidly dividing.

It is known that the synthesis of ribosomes parallels growth rate in most cells (reviewed in reference 21); therefore, the induction of ribosome synthesis can be accomplished by the addition of growth-stimulating factors to cells (30). We have demonstrated that in *Drosophila* cells that are able to divide, added serum also rapidly up-regulated rRNA synthesis. Similarly, in mammalian cultured cells, serum deprivation causes a down-regulation of Pol I transcriptional activity in vitro (6, 26, 28, 29). However, the addition of serum to the nondividing secretory cells did not stimulate rRNA production (32).

These alterations in rRNA synthesis do not appear to be regulated at the level of processing of the primary rRNA transcript. Changes in stability of the newly synthesized rRNAs could possibly account for the changes in rRNA accumulation that we observe. However, this is unlikely in view of the observed long half-life of rRNA in most cells. *Drosophila* larvae raised on rich growth medium show a half-life for rRNA of 48 h, while those raised on poor growth medium have a much longer rRNA half-life of 115 h (31). Others have investigated whether wastage of pre-rRNA is a mechanism used to control rRNA gene expression. For example, studies by Bowman (5) and Liebhaber et al. (13) have clearly shown that this mechanism is not used to regulate rRNA production in eukaryotic cells.

Allo et al. (1) reported that in cultured cardiomyocytes, phorbol esters like TPA increased both protein and rRNA accumulation. Consistent with our own observations, the increase in rRNA accumulation was not due to changes in processing of the pre-rRNA transcript. Similarly, the nonexpressing human rRNA genes in mouse-human hybrid cells can be activated by TPA (27). Therefore, these results represent another example that phorbol esters affect rDNA transcription under conditions associated with growth.

Recently Garber et al. (10) reported that the phorbol ester TPA induces specific expression of RNA Pol III-transcribed genes in *Drosophila* Schneider cells. This rapid transcriptional response to TPA can be reproduced in vitro, as nuclear extracts from TPA-treated cells exhibit increased transcription of 5S RNA and tRNA templates.

We have also studied the in vitro transcription of Pol I templates by using TPA- and serum-treated nuclear extracts from *Drosophila* Schneider cells (7a). The enhanced transcription that we observe following TPA or serum treatment is also observed in nuclear extracts of similarly treated cells.

In summary, results from nuclear run-on experiments demonstrate that TPA enhances rDNA transcription in dividing and nondividing *Drosophila* cells. Furthermore, in dividing cells, serum factors increase rDNA transcription.

Since rRNA synthesis represents the majority (>60%) of nuclear transcription, then even a twofold change in rDNA transcription represents a significant alteration in total nuclear transcription. The possible involvement of a protein kinase C-mediated pathway suggests an interesting mechanism by which regulation of rRNA gene expression in terminally differentiated or dividing *Drosophila* cells can be altered. Finally, the *Drosophila* system is one in which we can study rDNA regulation in the whole organism as well as in cultured cells. We now have the opportunity to dissect the molecular mechanisms of transcription by both genetic and biochemical means.

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