Is Apparent Autoregulatory Control of Tubulin Synthesis Nontranscriptionally Regulated?

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ABSTRACT Virtually all higher eucaryotic cells rapidly depress synthesis of new α- and β-tubulin polypeptides in response to microtubule inhibitors that increase the pool of depolymerized subunits. This apparently autoregulatory control of tubulin synthesis is achieved through modulation of tubulin messenger RNA levels. In particular, in cells treated with the microtubule-depolymerizing drug colchicine, tubulin messenger RNAs are specifically and rapidly lost from the cell cytoplasm. *A priori* this loss may be the result of suppression of new tubulin RNA transcription, failure of newly synthesized tubulin RNAs to be properly processed or transported from the nucleus, or an increased rate of cytoplasmic tubulin RNA degradation. Although transcriptional regulation has been demonstrated for most cellular eucaryotic genes thus far investigated in detail, we found that the apparent rates of tubulin RNA transcription were essentially unchanged in isolated nuclei derived from colchicine treated or control cells. This finding argues that the principal control of tubulin gene expression in response to altered subunit pools is probably not achieved through a transcriptionally regulated mechanism.

Although it seems clear *a priori* that the synthesis of cytoskeletal proteins must be correlated with cell growth and differentiation, the mechanism(s) through which this regulation is achieved has received little attention. Recently, however, a number of reports have demonstrated dramatic modulation of tubulin synthesis with cellular morphological changes (1, 3, 8, 27). In particular, the provocative report of Ben Ze'ev et al. (3) demonstrated that colchicine, which depolymerizes microtubules and raises the level of tubulin subunits in the cell (28), causes a cessation of tubulin synthesis within 6 h. In contrast, vinblastine, a drug that causes depolymerization followed by "precipitation" of the tubulin subunits (2, 5, 13) results in a small increase in new synthesis. Together, these observations suggest that the level of unpolymerized tubulin subunits modulates the level of new tubulin synthesis. We subsequently extended this initial observation to determine that the kinetics of the cellular response to a wide range of antitubulin drugs is rapid (with a half time of 1-1.5 h) and that this is a general phenomenon occurring in virtually all higher eucaryotic cells (8).

Conceptually, this apparent autoregulatory control of tubulin synthesis may be achieved through any of the following general molecular mechanisms: (*a*) translational sequestration or reversible inactivation of tubulin messenger RNAs (mRNAs),[1] (*b*) transcriptional modulation of tubulin genes,

(*c*) specific degradation of tubulin RNAs in the cytoplasm, or (*d*) inhibition of proper tubulin RNA processing/transport in the nucleus. To date, using cloned copies of α- and β-tubulin mRNA from chick brain (6), we have demonstrated that tubulin RNAs are specifically lost from the cell following colchicine treatment and hence that translational sequestration of intact mRNAs cannot be the mechanism responsible for controlling tubulin expression (8).

For most cellular genes thus far studied in detail in eucaryotes, control of expression has been demonstrated to be achieved primarily at the level of RNA transcription. For example, expression of globin (17, 21), ovalbumin (22, 23), and a variety of randomly selected RNAs from liver (10) are all regulated transcriptionally. In the present work we tested whether tubulin expression as a function of the apparent pool size of tubulin subunits is also modulated transcriptionally. We found that nuclei isolated from cells depleted in tubulin RNAs as the result of treatment with colchicine transcribed amounts of tubulin RNAs essentially indistinguishable from those of control cell nuclei. We concluded, therefore, that it is unlikely that the level of tubulin synthesis in response to fluctuations in subunit pool size is specified by a transcriptional control mechanism.

MATERIALS AND METHODS

Cell Culturing and Nuclei Isolation: Chinese hamster ovary (CHO) cells were cultured in plastic dishes in modified Eagle's medium α (Gibco Laboratories, Grand Island, NY) supplemented with 5% fetal calf serum

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and 5% calf serum. Human diploid fibroblasts (IMR-90) were also grown attached to petri dishes in Dulbecco's modified Eagle's medium containing 10% fetal calf serum.

Nuclei were isolated in essentially the same manner as described by Groudine et al. (17), and were stored at −80°C in 80-μl aliquots at an A^{260} of about 20.

Nuclear Transcription and RNA Isolation: Transcription of nuclei and subsequent RNA isolation were performed in 100-μl reactions by the method of McKnight and Palmiter (22) as modified by Groudine et al. (17). Typically, using [α-^{32}P]uridine triphosphate (UTP) of specific activity 400 Ci/mmol, 3–5 × 10^7 cpm were incorporated per reaction into trichloroacetic acid-precipitable material.

Recombinant Plasmids Carrying α-Tubulin, β-Tubulin, or Actin Sequences: Plasmids pT1, pT2, and pA1 carrying >95% of the coding sequences for embryonic chick brain α-tubulin, β-tubulin, and β-actin, respectively, were prepared as described previously (6). A recombinant plasmid, pkα1, containing the entire coding sequence of a human α-tubulin mRNA was provided by Dr. E. Fuchs (University of Chicago) from a complementary DNA (cDNA) library constructed from cultured human keratinocyte RNA (12). Similarly, a plasmid phβA carrying about 775 bases of sequence for human β-actin mRNA (372 nucleotides of coding sequence and 403 nucleotides of the 3'-untranslated region) was provided from the same library by Drs. E. Fuchs and I. Hanukoglu (19). Plasmid pDβ1 carrying essentially the complete coding sequence for a human β-tubulin mRNA was kindly provided by Drs. Nick Cowan (New York University) and Paul Dobner (University of Massachusetts Medical Center) (18).

Preparation of DNA Immobilized on Filters: Purified plasmid DNA containing cDNA specific for actin, α-tubulin, or β-tubulin was digested with a restriction endonuclease which cut only once within each plasmid. The DNAs were electrophoresed on 0.8% agarose gels and transferred to nitrocellulose in the manner described by Southern (26). The resultant filters were baked at 80°C for 3 h and prehybridized overnight in a solution of 50% formamide, 5× standard saline-citrate buffer (1× = 150 mM NaCl, 15 mM sodium citrate), 50 mM sodium phosphate, pH 7, 0.02% each of BSA, Ficoll 400 (Pharmacia Fine Chemicals, Inc., Piscataway, NJ), and polyvinylpyrrolidone, 50 μg/ml of sonicated, denatured *E. coli* or pBR322 DNA, and 250 μg/ml yeast RNA. Hybridization was performed in 1.5–2.5 ml of the same solution to which 5 × 10^5–1 × 10^7 cpm of probe RNA had been added. Filters were washed successively with four changes of 0.1% SDS and 2× standard saline-citrate buffer at room temperature followed by three washes for 20 min each in 0.1% SDS and 0.1× standard saline-citrate buffer, 53°C. Blots were wrapped in Saran Wrap and exposed at −80°C using Kodak XAR film and Dupont Lightning Plus intensifying screens (E. I. DuPont de Nemours & Co., Inc., Newtown, CT).

Quantitation of Blot Analyses: We quantitated the strength of hybridization of ^{32}P-labeled RNAs to immobilized DNA filters by densitometry of autoradiographic tracks using a densitometer (Kontes Co., Vineland, NJ) and a numerical integrator (Hewitt-Packard Co., Palo Alto, CA).

Preparation and Blotting of Cytoplasmic RNA: Cytoplasmic RNA from the supernatants of the nuclear isolation steps was prepared as previously described (8). Concentration was determined by A^{260} and the samples were then stored at −80°C. Gels for separation of RNA were poured from 1% agarose containing 2.2 M formaldehyde (4). RNA was transferred to nitrocellulose by the method of Thomas (29). Conditions of hybridization to ^{32}P-labeled cDNA probes were the same as previously described (8). Preparation of ^{32}P-probes was done according to Shank et al. (25).

RESULTS

Transcription from Isolated Nuclei: Conditions for DNA Excess and Saturation of Hybridization

To measure the specific transcription rates of α-tubulin, β-tubulin, and actin RNA sequences, we employed recombinant clones carrying tubulin or actin sequences copied from chicken or human mRNAs. As an assay for transcription of each of these genes, cloned DNAs were linearized, separated on agarose gels, blotted onto nitrocellulose, and hybridized to purified ^{32}P-labeled RNA synthesized in vitro from isolated nuclei. In this system, RNA chain initiation probably does not occur (17, 22). Thus, for the most part, the newly synthesized RNAs are transcribed by in vivo initiated RNA polymerase molecules already distributed along each transcription unit. Under the specific reaction conditions employed (see

Materials and Methods for details), CHO cell and human fibroblast nuclei incorporated labeled UTP into trichloroacetic acid-precipitable RNA at a constant rate during the first 30–40 min of incubation (data not shown). To investigate the sizes of the new RNA transcripts, we visualized the labeled RNA species by autoradiography following denaturing gel electrophoresis and autoradiography. As expected, they were found to be heterogeneous in size ranging from some abundant small species (about 100–200 nucleotides long) through a continuum of RNA lengths up to and exceeding the size of 28S ribosomal RNA (rRNA).

Initially, to insure that the signal detected for each of the RNAs analyzed corresponded quantitatively to the proportion of the total ^{32}P-labeled UTP incorporated into the homologous RNA species, it was necessary to identify conditions of hybridization that were in DNA excess and that were driven to saturation. Determination of these conditions is shown in Fig. 1, *A* and *B* for RNA transcribed from CHO and fibroblast cell nuclei. In Fig. 1*A*, filters containing increasing amounts of cloned DNA for α-tubulin or for actin were prepared and hybridized with identical labeled aliquots of runoff RNA transcripts. As is evident, above 0.1 μg of coding sequence for either α-tubulin or actin, the maximum hybridization was achieved. We concluded that under these conditions hybridization is essentially under conditions of DNA excess. Similarly, as shown in Fig. 1*B*, strength of signal increased with time of hybridization up to ~4 d, at which time intensity did not increase, thus establishing the length of hybridization required to achieve saturation.

That both of these conditions had in fact been obtained was further supported by rehybridization of the unbound RNA from one reaction mix to a second filter. Appropriate autoradiograms of an initial hybridization of in vitro transcribed CHO RNA to pBR322 (lane *1*), actin (lane *2*), α-tubulin (lane *3*), and β-tubulin (lane *4*) cDNAs and of the corresponding rehybridization experiment are shown in Fig. 1, *C* and *D*. No signal is detectable on the second filter.

Relative Transcription in Nuclei Isolated from Control Cells or from Colchicine-treated Cells

To analyze the relative rates of transcription of α-tubulin, β-tubulin, or actin RNAs in CHO cell nuclei or in CHO nuclei derived from cells containing elevated tubulin subunit levels induced by incubation in 10 μM colchicine for 6 h, we isolated in parallel nuclei from control and from colchicine treated cells. ^{32}P-labeled UTP was added to aliquots containing equivalent numbers of either sample of nuclei and transcription was allowed to proceed for 5–30 min at 26°C. RNA was then purified from each sample and hybridized to a filter containing α-tubulin, β-tubulin and actin cDNAs. Autoradiograms of the resultant filters are shown in Fig. 2, *A–I*. *A* and *B* represent hybridizations to cloned chicken cDNAs of RNA derived from 5-min incubations of control nuclei and colchicine nuclei, respectively. *C* and *D* represent the corresponding experiments with RNA derived from 30 min of in vitro transcription. Note that no obvious difference between the control and colchicine-derived RNA transcripts can be detected by the relative or absolute (the exposure times are all identical) numbers of counts hybridized to any of the three cloned cDNAs. This was true even at the shortest times of incubation. Quantitation of these results, displayed in Table I, confirmed this qualitative conclusion. Finally, *E* and *F* are duplicates of *C* and *D* showing hybridization of RNA gener-

ated from control and colchicine-treated nuclei prepared in an experiment parallel to but independent from those utilized in *C* and *D*. In this latter experiment, however, the human cDNA clones rather than the chicken clones were utilized as hybridization probes. Again, no significant differences in the
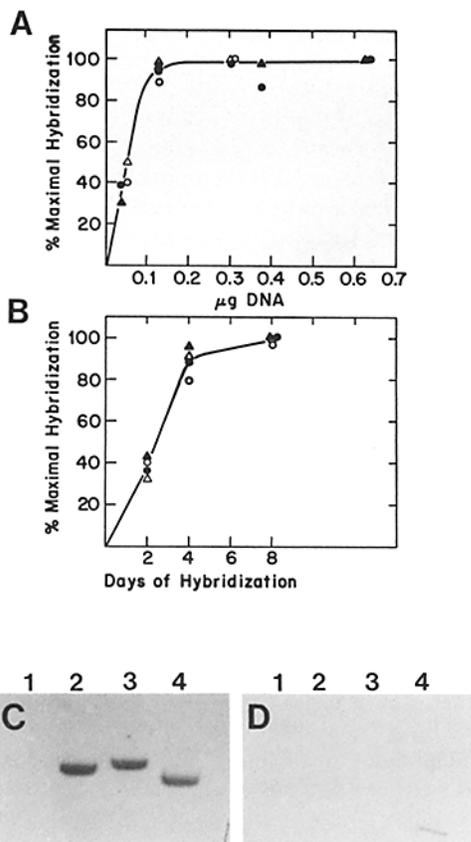

FIGURE 1 Determination of conditions of DNA excess and saturation of hybridization. Linearized, denatured plasmids carrying actin- or α-tubulin-specific sequences were bound to nitrocellulose filters according to Southern (24) and were hybridized against ^{32}P-labeled runoff RNA isolated from control cell nuclei. In *A*, replicate filters containing increasing amounts of DNA were hybridized for 4 d against equal aliquots of RNA (10^7 cpm), washed as described in Materials and Methods, and exposed to x-ray film. Intensity of hybridization was quantitated either by densitometry or by scintillation counting of the appropriate regions of the filters. In *B*, filters containing 0.15 μg of sequence-specific DNA were hybridized against 10^7 cpm of runoff RNA for increasing lengths of time up to 8 d. The filters were washed and then exposed together on a single piece of film. Intensity of hybridization was quantitated as before and the results are plotted relative to the strongest signal observed for each plasmid sample. Open symbols are the results of hybridizations with CHO runoff RNA to immobilized chicken cDNA clones; closed symbols are from human fibroblast RNA to immobilized human cDNA clones. (O) α-tubulin hybridization; (Δ) actin hybridization. In *C*, an autoradiogram of a filter containing 0.15 μg of sequence-specific DNA for pBR322 (lane *1*), actin (lane *2*), α-tubulin (lane *3*), and β-tubulin (lane *4*) is shown following hybridization for 4 d with 10^7 cpm of runoff RNA from CHO cells. *D* displays an autoradiograph of a corresponding filter which was hybridized for an additional 4 d against the unhybridized RNA recovered from *C*. The two filters were exposed together following the second hybridization. Note that no signal can be detected in this rehybridization experiment, thus verifying that the initial hybridization was carried to completion.
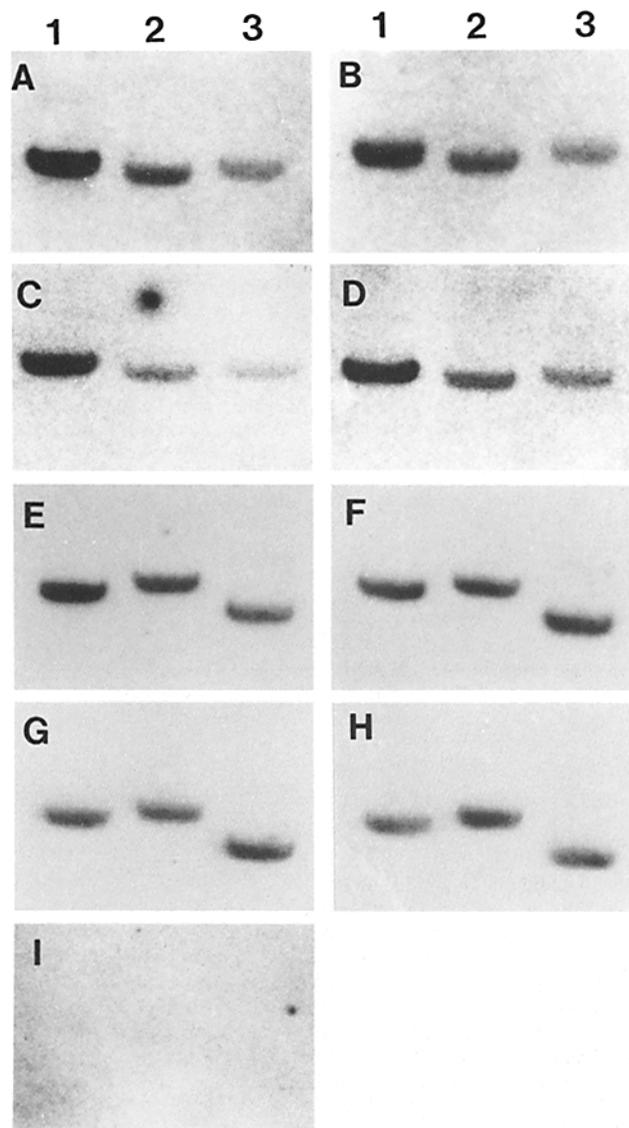

FIGURE 2 Relative transcription in CHO nuclei isolated from control cells or from colchicine-treated cells. ^{32}P-labeled runoff RNA from nuclei of control or colchicine-treated cells was hybridized for 4 d to filters containing 0.5 μg of sequence-specific cloned chicken cDNA for (lane *1*) actin, (lane *2*) α-tubulin, and (lane *3*) β-tubulin. The filters were then washed as described in Materials and Methods, and exposed to x-ray film. An autoradiogram of the resultant films is shown. *A* and *B* represent hybridization of 5 × 10^6 cpm of RNA derived from 5 min of in vitro transcription of control and colchicine-treated nuclei, respectively. *C* and *D* display the corresponding experiments with RNA derived from 30 min of in vitro transcription. The specific activity of the [α-^{32}P]UTP utilized for *A–D* was 400 Ci/mmol. *E* and *F* display experiments homologous to those shown in *C* and *D* with the control and colchicine-derived nuclei, with the following exceptions: (a) the nuclei utilized were prepared independent from those in *C* and *D*, (b) the human cDNA clones were employed as hybridization probes in place of the chicken clones, and (c) the specific activity of the in vitro transcribed RNA was six times higher as the result of utilization of [α-^{32}P]UTP of 2400 Ci/mmol. *G* and *H* represent the duplicates of *E* and *F*, except that the in vitro runoff reactions were carried out in the presence of 1 mg/ml of heparin. Finally, *I* displays the hybridization pattern of 10^7 cpm of in vitro transcribed RNA isolated from control CHO nuclei to which α-amanitin had been added to a final concentration of 2 μg/ml.

TABLE I

Quantitation of Apparent Relative Rates of Transcription of Actin and Tubulin*

	CHO cell nuclei (transcripts measured using the chicken cDNA clones)			CHO cell nuclei (transcripts measured using the human cDNA clones)			Human fibroblast nuclei	
	Actin	α-Tubulin	β-tubulin	Actin	α-Tubulin	β-Tubulin	Actin	α-Tubulin
Control nuclei transcribed in vitro for 30 min	100[‡]	34 (5)[‡]	27 (6)[‡]	100	105 (9)	105 (8)	100	49 (10)
Control nuclei transcribed in vitro for 5 min	100[§]	25 (8)[§]	13 (4)[§]				100	45 (7)
Nuclei from colchicine treated cells, transcribed 30 min	100[‡]	40 (4)[‡]	33 (6)[‡]	100	100 (8)	93 (10)	100	39 (7)
Nuclei from colchicine treated cells, transcribed 5 min	100[§]	41 (6)[§]	23 (6)[§]				100	40 (9)

* Data derived from densitometric scans of in vitro runoff RNAs hybridized to immobilized cDNAs.
All data points represent a minimum of two separate measurements and have been normalized to set the actin signal at 100%. Standard errors are given in parentheses.
[‡] Average of experiments from three independent preparations of nuclei.
[§] Average of experiments from two independent preparations of nuclei.
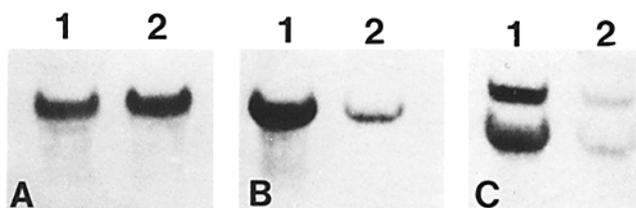

FIGURE 3 Measurement of cytoplasmic actin, α-tubulin, and β-tubulin RNAs in control and colchicine-treated cells. Total cytoplasmic RNA in the postnuclear extracts from the nuclei preparations used for the in vitro transcription analyzes shown in Fig. 2, A–D was isolated, and equal amounts of RNA (10 μg) were electrophoresed on a denaturing gel. After transfer to nitrocellulose, actin, α-tubulin, or β-tubulin RNAs were detected by hybridization to the appropriate ³²P-labeled probes. Autoradiograms of those hybridizations are shown. Lane 1 in each part represents RNA from control cell cytoplasm; lane 2 represents RNA from colchicine-treated cell cytoplasm. (A) hybridization to an actin probe; (B) hybridization to an α-tubulin probe; (C) hybridization to a β-tubulin probe. Note the dramatic loss of tubulin RNA sequences with respect to total RNA or to the actin RNA level.

relative or absolute hybridization intensities are evident for actin, α-tubulin or β-tubulin (compare E and F). These data clearly support the argument that tubulin transcription rates in nuclei derived from colchicine-treated CHO cells are essentially indistinguishable from those in control nuclei.

Additional evidence for this conclusion was obtained from in vitro transcription reactions containing sarkosyl or heparin (at 0.5% or 1 mg/ml, respectively). As discussed by Groudine et al. (17), these two compounds remove histones and most other chromosomal proteins, but leave initiated RNA polymerases still bound to DNA and capable of elongation but not new initiation (11, 14–16). An example of an experiment using RNAs transcribed in the presence or absence of heparin is shown in Fig. 2, G and H, respectively, for transcripts produced by control and colchicine-treated CHO nuclei. As before, no substantial differences between the colchicine or control nuclei hybridization patterns could be observed either when compared with each other or when compared with the corresponding heparin-free transcription patterns (Fig. 2, E and F).

Cytoplasmic Tubulin RNA Sequences Are Dramatically Depressed by Colchicine Treatment, even though Tubulin Transcription Rates Are Apparently Unaltered

In view of the failure to detect a measurable difference in the runoff transcription products from control and colchicine-derived nuclei, one possible explanation was that the colchicine-treated cells had not in fact suppressed new tubulin synthesis. We showed that this was not the case in the following manner. Cytoplasmic RNA was purified from the postnuclear CHO cell extracts obtained during the nuclei preparation. Equal amounts of control cell and colchicine-treated cell RNA were electrophoresed on an agarose gel, transferred to nitrocellulose, and hybridized against an actin probe, an α-tubulin probe, or a β-tubulin probe. Autoradiograms of the resultant fiters are shown in Fig. 3, A–C. As expected, actin RNA levels were essentially equivalent in control and drug-treated cells. Both α- and β-tubulin RNAs, on the other hand, were dramatically reduced in the colchicine-derived sample. From quantitation of the autoradiographs by densitometry, we found that <10% of the initial amount of α- or β-tubulin RNAs remained after 6 h of colchicine treatment. Therefore, as we originally reported (8), CHO cells treated with colchicine had indeed lowered tubulin mRNA levels by greater than 10-fold even though the apparent in vitro transcription rates for new tubulin RNAs remained unaltered.

Hybridization Signals Detected for Actin and Tubulin Are Authentic Products of RNA Polymerase II

Implicit in the runoff transcripton assay shown in Fig. 2 is the assumption that the hydridization signals detected represent true transcription by RNA polymerase II of tubulin and actin gene sequences. To demonstrate that, in all probability, the signals observed are derived from RNA polymerase II transcription, we performed runoff reactions using control or colchicine-derived nuclei in the presence of 2 μg/ml of α-amanitin. This level of α-amanitin is sufficient to fully inhibit RNA polymerase II (20), and, in the present experiment, resulted in a 50% decrease in the incorporation of labeled

UTP into new RNA. However, it also resulted in the loss of all radioactivity hybridizable to immobilized actin or tubulin DNAs (Fig. 2*I*). As expected, ribosomal RNA synthesis, as judged by hybridization to pX1r101, a plasmid that carries the 18 and 28S ribosomal RNA genes of *Xenopus laevis* (24), was not altered by the presence of the drug (data not shown).

Relative Rates of Tubulin and Actin Transcription in Human Fibroblast Nuclei

To determine whether the lack of apparent transcriptional regulaton was also seen in cell types additional to CHO cells, runoff transcription experiments were performed in nuclei from human diploid fibroblast cells that had been pretreated for 0 or 6 h in 10 μM colchicine. Quantitation of the hybridizations to actin or to α-tubulin after 5 or 30 min of in vitro transcription is given in Table I. Essentially indistinguishable levels of tubulin transcripts were produced in control and colchicine-treated nuclei. Again, the relative cytoplasmic levels of tubulin RNAs present in the cytoplasm of control and colchicine-treated cells were analyzed by RNA blots. Both α- and β-tubulin RNAs were found to be specifically depressed by a factor of four in the RNA from the colchicine-treated cells, whereas actin mRNA levels were unaltered (data not shown).

DISCUSSION

By employing runoff transcription of isolated nuclei and subsequent hybridization of the in vitro synthesized [^{32}P]-RNA to fragments of cloned tubulin or actin DNAs under conditions of DNA excess and saturation of hybridization, we found that the fraction of new nuclear RNA which was transcribed for α- or β-tubulin gene sequences was unaffected by pretreatment of cells with a microtubule inhibitor which causes microtubule depolymerization. If we employ the likely assumption that the cytoplasmic appearance of new tubulin RNAs can be described by a single rate constant of nuclear transcription/processing/transport and that the cytoplasmic rate of mRNA degradation can be described by a first-order decay process, then, in order for a transcriptional control mechanism to achieve a specified lowering of the cytoplasmic RNA level, there must be a proportional reduction in the transcription/processing/transport rate. In the present experiments, although the cytoplasmic levels of both α- and β-tubulin RNAs (but not actin RNAs) were reduced 4- to 12-fold by colchicine treatment, the specific in vitro transcription rates appeared to be largely unaffected. Therefore, these data suggest that transcriptional regulation of tubulin genes is not the principal mechanism by which tubulin expression is modulated in response to fluctuations in the depolymerized subunit pool size. This tentative conclusion requires that the following additional parameters be considered.

First, the validity of this conclusion initially rests on the assumption that the in vitro transcription system faithfully represents the in vivo situation and that changes in in vivo transcription rates can be detected with the in vitro assay. This certainly is the case for a variety of genes previously studied. For example, using nascent transcripts from isolated nuclei, McKnight and Palmiter (22) demonstrated dramatic transcriptional regulation of ovalbumin in the chick oviduct in response to estrogen or progesterone stimulation. Moreover, a two- to threefold induction of conalbumin was also measured in response to hormone treatment. Similarly, Grou-

dine et al. (17) and Landes et al. (21) have demonstrated that the switching from embryonic to adult globin synthesis in chick erythrocytes is transcriptionally modulated. In addition, Derman et al. (10) have used isolated nuclei to show transcriptional regulation of a variety of genes expressed in liver.

Second, it is possible that the failure to detect a substantial difference in the rate of tubulin transcription in control or colchicine-treated cells is a consequence of loss of important regulatory factors during nuclear isolation. Given that the in vitro system produces RNAs transcribed essentially exclusively by RNA polymerase molecules initiated in vivo, this exciting possibility would require the in vivo presence of a negative regulatory molecule which acts not to block polymerase initiation but rather to block polymerase elongation. We have tried to test the obvious possibility that tubulin subunits themselves might be such regulatory molecules by adding purified tubulin polypeptides to runoff transcription reactions. To date, such additions have produced no measurable difference in tubulin transcription (data not shown). Moreover, we are in the process of attempting to deterine with in vivo [^{3}H]uridine labeling of newly synthesized nuclear RNAs whether transcription of tubulin RNAs in vivo is affected by colchicine treatment. If transciption is again found to be unchanged, then transcriptional control can reasonably be excluded as the primary regulatory mechanism through which tubulin synthesis is modulated by fluctuations in the subunit pool size.

We should also note that not only is no diminution in tubulin synthesis relative to actin apparent in nuclei from colchicine-treated cells but also the data (Fig. 2 and Table I) indicate a mild increase in tubulin transcription. Given the small fraction of the total labeled RNA expected in tubulin specific sequence (ribosomal RNA and transfer RNA synthesis account for the majority of labeled RNA) and the resultant, unavoidable scatter in the data, this apparent increase is probably not significant. We are confident, however, that measurement of the dramatic decrease (>4- to 12-fold) in tubulin synthesis which would be necessary for loss of tubulin sequences solely through transcriptional modulation following colchicine treatment would be well within the sensitivity of the transcription/blot assay.

The inhibition of all new RNA transcription by treatment of cells with actinomycin D has been previously shown to cause a loss of tubulin RNAs and synthesis of tubulin polypeptides with a half time that is indistinguishable from that observed by treatment of the cells with colchicine (7). Moreover, simultaneous treatment of cells with both drugs also yields loss of tubulin synthesis with identical kinetics. These observations seem to suggest that tubulin synthesis is modulated through a nuclear event during colchicine treatment, since the combination of an independent cytoplasmic effect with the actinomycin-induced transcriptional shutoff would result in an accelerated loss of tubulin sequences in the double-drug-treated cells. If this is correct, then the current data combined with our previous work (7–9) and with that of Ben Ze'ev et al. (3) collectively suggest that tubulin synthesis is probably modulated by the apparent pool size of depolymerized subunits through a nuclear mechanism which may involve either a nuclear inhibition of processing/transport of tubulin RNAs or a negative regulator of transcription that is not maintained in isolated nuclei.

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