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# Gene-Specific Signal Transduction between Microtubules and Tubulin Genes in *Tetrahymena thermophila*

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**Mammalian cells regulate tubulin mRNA abundance by a posttranscriptional mechanism dependent on the concentration of tubulin monomer. Treatment of mammalian cells with microtubule-depolymerizing drugs and microtubule-polymerizing drugs causes decreases and increases in tubulin mRNA, respectively (D. W. Cleveland, Curr. Opin. Cell Biol. 1:10–14, 1989). In striking contrast to the case with mammalian cells, perturbation of microtubules in *Tetrahymena thermophila* by microtubule-depolymerizing or -polymerizing drugs increases the level of the single α-tubulin gene message by increasing transcription (L. A. Stargell, D. P. Heruth, J. Gaertig, and M. A. Gorovsky, Mol. Cell. Biol. 12:1443–1450, 1992). In this report we show that antimicrotubule drugs preferentially induce the expression of one of two β-tubulin genes (*BTU1*) in *T. thermophila*. In contrast, deciliation induces expression of both β-tubulin genes. Tubulin gene expression was examined in a mutant strain created by transformation with an in vitro-mutagenized β-tubulin gene that conferred resistance to microtubule-depolymerizing drugs and sensitivity to the polymerizing drug taxol and in a strain containing a nitrosoguanidine-induced mutation in the single α-tubulin gene that conferred the same pattern of drug sensitivities. In both cases the levels of tubulin mRNA expression from the drug-inducible *BTU1* gene in the mutant cells paralleled the altered growth sensitivities to microtubule drugs. These studies demonstrate that *T. thermophila* has distinct, gene-specific mechanisms for modulating tubulin gene expression depending on whether ciliary or cytoplasmic microtubules are involved. They also show that the cytoplasmic microtubule cytoskeleton itself participates in a signal transduction pathway that regulates specific tubulin gene transcription in *T. thermophila*.**

The cytoskeleton has long been known to play a fundamental role in cell shape, in intracellular movement, in cell motility, in cell division, and in maintenance of the internal organization of the cell. The cytoskeleton also appears to be involved in the posttranscriptional regulation of gene expression. A number of lines of evidence suggest that the cytoskeleton is involved in the establishment and maintenance of nonuniform distribution of mRNAs that may be important in regulating the sites or the levels of translation of specific messages (for reviews see references 5, 24, 43, and 47). Morphological and cell fractionation studies indicated that both actin filaments and microtubules are often physically associated with mRNA molecules or with polyribosomes (5, 47), and inhibitors that depolymerize either microfilaments or microtubules have been shown to affect the localization of mRNAs in oocytes (39, 40, 50).

The best-understood example of the role of the cytoskeleton in the posttranscriptional regulation of gene expression concerns the autoregulation of tubulin synthesis. Microtubules are dynamic polymers composed mainly of heterodimers of α- and β-tubulin polypeptides (see references 31 and 32 for reviews). In vitro studies suggest that polymerized microtubules and tubulin monomers are in a complex, dynamic equilibrium in which the monomer concentration can influence the number, length, and stability of microtubules (29). Cultured mammalian cells have a mechanism in which the monomer concentration is autoregulated by changing the amount of tubulin mRNA in response to changes in the amount of tubulin monomer (6, 10–12). An increase in the level of tubulin monomer caused by treatment with microtubule-depolymerizing drugs or by microinjection of tubulin results in a rapid decline in tubulin synthesis. Detailed analyses by Cleveland and colleagues (for a recent summary see reference 2) of the mechanisms underlying this autoregulation demonstrated that the tubulin subunits stimulated specific cotranslational degradation of tubulin mRNA. The sequence (MREI) required for the autoregulation of β-tubulin mRNA is the first four highly conserved amino acids (49). Since tubulin subunits do not bind directly to the MREI sequence, another cellular component is likely to be involved (48). This hypothetical factor would have to increase in concentration or activity coordinately with tubulin monomer and bind to the MREI nascent polypeptide to somehow specifically stimulate tubulin mRNA degradation. The mechanism underlying α-tubulin regulation, while also cotranslational, is less well understood and differs in detail from that regulating β-tubulin expression (2).

In contrast to the numerous lines of evidence suggesting involvement of the cytoskeleton in posttranscriptional regulation of gene expression, there is little evidence of its direct involvement in the regulation of transcription itself. Thus, it was somewhat surprising that when we treated the ciliated protozoan *Tetrahymena thermophila* with microtubule-depolymerizing drugs such as oryzalin and colchicine, it responded in a manner quite different from that of mammalian cells (45). Instead of decreasing tubulin synthesis and tubulin mRNA levels by degrading tubulin messages, *T. thermophila* responded to microtubule depolymerization by increasing α-tubulin synthesis and tubulin mRNA levels by increasing α-tubulin gene transcription without any detectable change in message turnover. Equally surprising was the fact that taxol, a

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microtubule-polymerizing agent, had the same effect as microtubule-depolymerizing agents in *T. thermophila*. These studies suggested that *T. thermophila* responded to any perturbation in the microtubule cytoskeleton by increasing α-tubulin gene transcription. However, it could be argued that the effects were nonspecific responses to the drugs rather than results of a specific interaction between the microtubules and the transcriptional apparatus.

In this report we extend our analysis of the effects of microtubule drugs to the two β-tubulin genes of *T. thermophila* (which encode identical proteins) and analyze the responses of mutant cells containing tubulin genes conferring altered sensitivities to microtubule drugs. These studies demonstrate that *T. thermophila* has at least two distinct pathways regulating tubulin gene transcription. One pathway must sense the loss of cilia per se. The other must operate through the cytoplasmic microtubule system, demonstrating for the first time that the cytoskeleton can play a specific role in regulating transcription.

## MATERIALS AND METHODS

**Strains and culture conditions.** Strains CU428 [*Mpr*/*Mpr*, *Pm*$^+$/*Pm*$^+$ (mp-s, pm-s, mating type VII)] and B2086 [*Mpr*$^+$/*Mpr*$^+$, *Pm*$^+$/*Pm*$^+$ (mp-s, pm-s, mating type II)] were kindly supplied by P. J. Bruns (Cornell University, Ithaca, N.Y.). Cells were grown in SPP medium (22) containing 1% Proteose Peptone. Cells were starved at a density of $1 \times 10^5$ to $2 \times 10^5$/ml in 10 mM Tris (pH 7.4) for 18 to 22 h at 28 or 38°C without shaking.

Strain VB1 was created by replacing the endogenous macronuclear *BTU1* β-tubulin genes with the *BTU1M*$^{350}$ gene, which encodes a single amino acid replacement (Lys-350 to Met-350) that confers oryzalin and vinblastine resistance and hypersensitivity to taxol (20). Gene replacement was accomplished by electroporation as described previously (20) except that 30 μM vinblastine was used instead of oryzalin in the initial selection. Fifteen micrograms of plasmid pH4T2 was coelectroporated with 50 μg of linearized pBTU1M$^{350}$. Plasmid pH4T2 contains the 5′ region of the *Tetrahymena H4-I* gene, a *TN5 Aph* gene which confers paromomycin resistance (28), and the 3′ region of the *Tetrahymena BTU2* gene (19). Gene replacement transformants were selected and confirmed by Southern blot analyses using an oligonucleotide probe specific for the mutated sequence as described previously (20).

Strain E5 was isolated by David Pennock (Miami University, Oxford, Ohio). It is a nitrosoguanidine-induced mutant that was selected on the basis of oryzalin resistance and taxol hypersensitivity. In a study to be described elsewhere (19a), it was shown to encode a single amino acid replacement (Ala-65 to Thr-65) in its single α-tubulin gene. E5 has been reverted to wild-type drug sensitivities by microinjection-mediated gene replacement with a wild-type α-tubulin gene to give strain AG1.

**Deciliation and drug treatment.** Cells were deciliated and incubated for regeneration of cilia as described previously (8) except that shaking was omitted during regeneration. Stock solutions of 100 mM oryzalin (a gift of David Pennock, Miami University, Oxford, Ohio) and of 20 mM taxol (Sigma) in dimethyl sulfoxide were used to make dilutions to the final concentrations indicated in Results. Drug treatments were performed at 38°C, except for those involving conjugation, which were done at 30°C, the temperature at which conjugation is normally performed (35). Control cells were mock treated with dimethyl sulfoxide.

**Whole-cell slot blots.** Samples containing $2 \times 10^5$ cells were removed at various times after drug treatment, and extracts of whole-cell mRNA were prepared, blotted, hybridized, and analyzed by a slight modification (45) of the method of Grimes et al. (23). DNA probes were labeled with [α-$^{32}$P]dATP by random hexamer priming (15). The templates used were JG-1, a genomic clone containing 1 kb of the *Tetrahymena pyriformis* β*TT1* gene coding sequence (4, 21), and p539-2, which contains a 250-bp fragment from the 3′ end of the *Tetrahymena* actin gene (13) (kindly provided by Ronald Pearlman, York University, North York, Ontario, Canada).

**RNase protection assays.** Total cell RNAs were extracted as described previously (9). RNase protection assays were performed as described previously (1) except that 4 μg of RNase A per ml and 90 U of RNase T1 per ml were used and the digestion was carried out on ice for 10 min. Hybridization was performed with a radiolabeled antisense probe made by in vitro transcription using T3 RNA polymerase (Boehringer Mannheim) as described previously (1). The template was cDNA clone pBTU1-24 containing the *BTU1* gene in Bluescript which had been truncated by *Xmn*I restriction endonuclease digestion 305 nucleotides upstream of the translation stop codon. Unincorporated nucleotides were separated from the probe by using a G-50 Quick-spin column (Boehringer Mannheim).

**Run-on transcription.** Run-on transcriptions with isolated nuclei were performed as described previously (46). One microgram of each of the following plasmids was linearized and immobilized on nitrocellulose by using a slot blot manifold: HE-1, a clone containing 20 bp of the coding region and 1.4 kb of the 3′ flanking region of the *BTU1* gene (21); BB-2, a clone containing 1.4 kb of the 3′ flanking region of the *BTU2* gene (21); pI210, a clone containing a 1.1-kb *Eco*RI-*Taq*I fragment of the *T. thermophila H4-I* gene (3); p539-2, containing the *T. thermophila* actin gene fragment (see above); and pTUB-6, a 1,162-bp fragment of which 718 bp encodes the 3′ end of the *T. thermophila* α-tubulin gene (33).

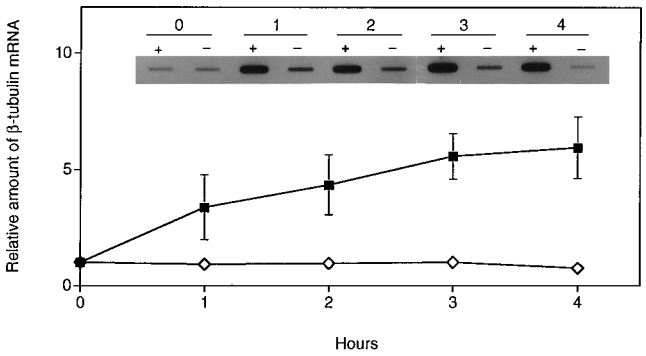


FIG. 1. Effects of oryzalin on β-tubulin mRNA accumulation in starved *T. thermophila* cells. After starvation for 18 h, the cultures were treated (+ and ■) or not treated (− and ◇) with oryzalin (7.5 μM). Samples were taken at 0, 1, 2, 3, and 4 h, and β-tubulin message levels were measured by total cell extract slot blot hybridization using a DNA probe derived from a 1-kb β-tubulin coding fragment. Slots were cut out, β-tubulin message levels were quantitated in a liquid scintillation counter, and the β-tubulin message level relative to that at 0 h was plotted. Values shown are the means and standard deviations for three experiments. Missing error bars are completely contained within the datum points. The inset shows an autoradiograph of a slot blot with extract equivalent to 3,000 cells per time point.

**Analysis of pair formation and nuclear retention.** Strains CU428 and B2086 were starved for 18 to 22 h at 30°C and then mixed at a concentration of $3 \times 10^5$ cells per ml. Cells were treated with oryzalin at various concentrations immediately after mixing. At 8 h after mixing, the percentage of cells in mating pairs was determined. At that time single pairs were also isolated by hand; they were allowed to grow in SPP for 2 to 3 days and then tested for resistance to 6-methylpurine. The phenotype of *T. thermophila* is determined by the genotype of the transcriptionally active macronucleus. Since macronuclei of both strains are 6-methylpurine sensitive, conjugants that retain their old macronuclei will be sensitive to the drug. On the other hand, conjugants that successfully complete conjugation form new macronuclei that are heterozygous progeny derived from the micronuclei of both parents. Since CU428 cells are heterokaryons that are homozygous for resistance to 6-methylpurine in their micronuclei, successful conjugants will be resistant to the drug.

## RESULTS

**The microtubule-depolymerizing agent oryzalin induces β-tubulin mRNA accumulation in starved *T. thermophila* cells.** Previous studies had shown that when starved *T. thermophila* cells were treated with 7.5 μM oryzalin (a concentration shown to depolymerize microtubules in vivo and the lowest concentration that inhibits both growth and cilium regeneration), α-tubulin mRNA is rapidly induced (45). Note that starved cells were used in these studies because they have greatly reduced levels of tubulin mRNA, making the induction easier to detect, and that starvation is a physiological state that is a prerequisite for conjugation. We performed similar studies to determine whether β-tubulin mRNA accumulates like α-tubulin mRNA. As seen in Fig. 1, oryzalin causes an approximately fivefold increase in the amount of β-tubulin message per cell, similar to the induction observed for α-tubulin. The amount of actin message in oryzalin-treated cells remained unchanged (data not shown). Thus, α- and β-tubulin messages are specifically and coordinately induced by treatment with this microtubule-depolymerizing drug.

**Only one of the two *Tetrahymena* β-tubulin genes is induced by oryzalin.** *T. thermophila* contains two β-tubulin genes, *BTU1*

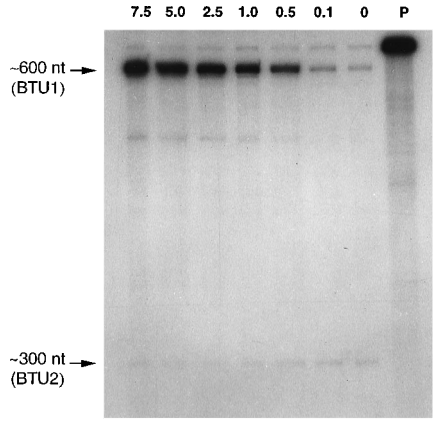


FIG. 2. Oryzalin dosage dependence of β-tubulin mRNA accumulation. Starved *T. thermophila* cells were treated with oryzalin for 2 h at different concentrations (micromolar) as indicated above each lane. Total cell RNA was extracted, and the abundance of β-tubulin message was determined by an RNase protection assay. P, undigested probe. Sizes of the fragments protected by the BTU1 and BTU2 messages are indicated by arrows. nt, nucleotides.

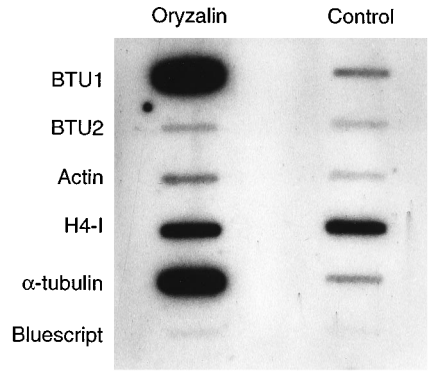


FIG. 3. Effect of oryzalin on tubulin transcription in starved *T. thermophila* cells. The transcription rate for each β-tubulin gene was measured by an in vitro nuclear run-on assay. Nuclei were isolated 2 h after treatment with oryzalin (7.5 μM) and incubated in transcription buffer containing [$^{32}$P]UTP for 30 min. Levels of transcription of specific genes were measured by hybridization of labeled transcripts with a slot blot containing different plasmid DNAs as indicated.

and *BTU2*, that encode the same protein (21). While the coding regions of the two genes are 96% identical, the 3′ untranslated regions show no obvious similarity, enabling the preparation of gene-specific probes that do not cross-react on Southern blots (data not shown) and that were used to develop a gene-specific RNase protection assay. In brief, a $^{32}$P-radiolabeled antisense riboprobe was synthesized by using the *BTU1* gene as a template. The 5′ 305 nucleotides of this probe correspond to the 3′ portion of the *BTU1* coding region, which is highly conserved (six mismatches) between the two genes. The 3′ 314 nucleotides of this probe correspond to the 3′ untranslated region of *BTU1* and are specific for this gene. After hybridization with total cell RNA and RNase digestion, the probe yields two major fragments of 619 and 305 nucleotides whose abundances correspond to the levels of *BTU1* and *BTU2* messages, respectively. We used this assay to study the expression of the two β-tubulin genes.

The intensities of the two gene-specific bands were similar in growing cells (data not shown) and starved vegetative cells (Fig. 2). When starved cells were treated with oryzalin, the level of *BTU1* message increased markedly while the level of *BTU2* message remained largely unchanged (Fig. 2). Thus, the abundances of the transcripts from the two genes can be independently regulated.

To determine whether the independent regulation of the two genes occurred at the transcriptional level, a nuclear run-on assay was performed (Fig. 3). As described previously (45), oryzalin greatly induced the transcription of the single *T. thermophila* α-tubulin gene, while the transcription rate of the actin gene increased only slightly. The transcription of *BTU1*, but not *BTU2* (or the histone H4-I gene), was markedly induced by oryzalin treatment, indicating that the gene-specific induction of β-tubulin mRNA accumulation is regulated at the transcriptional level.

**Similar concentrations of oryzalin affect *BTU1* transcription and other microtubule-mediated processes.** Figure 2 indicates that the accumulation of the *BTU1* message occurred at low doses (0.1 μM) of oryzalin and that it occurred in a dose-dependent manner. Since much higher doses of oryzalin (7.5 μM) are required to inhibit growth and cilium regeneration in *T. thermophila* completely, we wished to determine whether these lower drug concentrations would affect any physiological processes likely to be microtubule mediated. A number of microtubule structures are formed transiently during conjugation, the sexual stage of the life cycle of *T. thermophila* (17, 34). During conjugation, cells pair and the micronucleus undergoes meiosis, producing four haploid products, three of which later break down. The fourth undergoes a haploid mitosis producing two gametic nuclei that participate in reciprocal exchange and fertilization. The zygotic nucleus then divides twice to produce four nuclei, two of which develop into new macronuclei and two of which become new micronuclei. When new macronuclei develop, the old one is destroyed. If the new macronuclei fail to form properly, the old one is retained, or the exconjugants die. Thus, successful conjugation depends on numerous nuclear divisions and nuclear migrations that involve a variety of microtubule systems. Both pairing and macronuclear development have been shown to be sensitive to the effects of microtubule drugs (26, 27), and the drug dependence of both of these processes can be assayed (see Materials and Methods). As seen in Fig. 4, both pair formation and the level of macronuclear retention are affected in a dose-dependent manner by

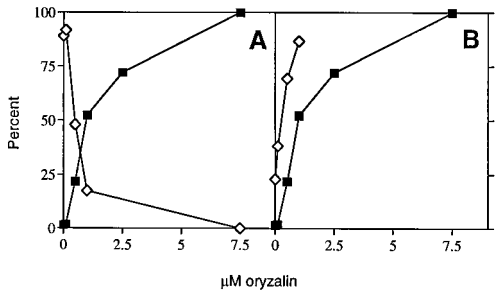


FIG. 4. Effects of oryzalin on pairing (A) and macronucleus retention (B) of conjugating *T. thermophila* cells. Cells of different mating types were starved and mixed together at different oryzalin concentrations. Eight hours later, the percentage of cells in mating pairs was determined and plotted (A) (◇). At the same time, 48 mating pairs were isolated for each concentration of oryzalin and the percentage of mating pairs retaining their old nuclei was determined (B) (◇). The nuclear retention rate at oryzalin concentrations higher than 1 μM could not be measured because pairing was almost completely inhibited. For comparison, the densities of the β-tubulin bands in an RNase protection assay of RNA from cells treated for 2 h with different oryzalin concentrations at 30°C were determined by cutting out the bands and counting them in a scintillation counter. The message levels relative to that in 7.5 μM oryzalin-treated cells were plotted (■).

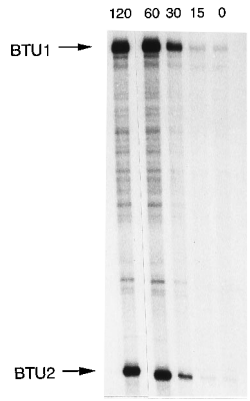


FIG. 5. Effect of deciliation on β-tubulin mRNA accumulation. Total cell RNA was extracted from starved *T. thermophila* cells at different times after deciliation as indicated above each lane (in minutes). β-Tubulin message abundance was analyzed by RNase protection.

concentrations of oryzalin that induce the accumulation and transcription of *BTU1* mRNA. Thus, the effects of oryzalin on *BTU1* gene expression occur at drug concentrations that also affect other cellular processes likely to involve cytoplasmic microtubules, consistent with the conclusion that the effects of oryzalin on the *BTU1* gene are mediated by the cytoplasmic microtubule cytoskeleton.

**Deciliation induces both *BTU1* and *BTU2* mRNA accumulation and transcription.** Deciliation has also been shown to induce tubulin mRNA accumulation and transcription in *Tetrahymena* spp. (42, 44; see below). To test the possibility that only *BTU1* is inducible, while *BTU2* is constitutively expressed, the mRNA levels were analyzed by RNase protection at various times after deciliation of starved cells. Figure 5 shows that messages from both genes accumulate within 15 to 30 min after deciliation as cilia regenerate. Nuclear run-on assays show that the induction of both genes is accompanied by increased transcription (data not shown). These results demonstrate that both *BTU1* and *BTU2* are inducible and suggest that deciliation and treatment with antimicrotubule drugs stimulate tubulin gene transcription by different mechanisms.

**Transformed cells containing a β-tubulin gene conferring oryzalin resistance show decreased induction of *BTU1* mRNA.** *BTU1M*$^{350}$ is an in vitro mutagenized version of the *BTU1* gene in which the codon for Lys-350 has been changed to Met. When this gene is transformed into *T. thermophila* cells by gene replacement (20), it confers resistance to oryzalin and other microtubule-depolymerizing drugs and hypersensitivity to the microtubule-stabilizing drug taxol. A mutant gene of *Chlamydomonas reinhardtii* (30) having this replacement confers a similar phenotype and also results in increased acetylation of α-tubulin, suggesting that this mutation results in stabilized microtubules (41). If oryzalin induction of *BTU1* mRNA accumulation is mediated through the microtubule cytoskeletal system, *T. thermophila* cells transformed with *BTU1M*$^{350}$ should require higher concentrations of oryzalin to induce *BTU1* mRNA accumulation.

We created *T. thermophila* VB1 by replacing the endogenous *BTU1* gene with *BTU1M*$^{350}$. This strain was also cotransformed with plasmid pH4T2, which confers neomycin resistance (see Materials and Methods). The RNase protection assay showed that induction of *BTU1* mRNA accumulation in strain VB1 was less sensitive to oryzalin treatment than induction in strains transformed with pH4T2 alone (Fig. 6). Note that the *BTU2* message served as an internal control in this experiment

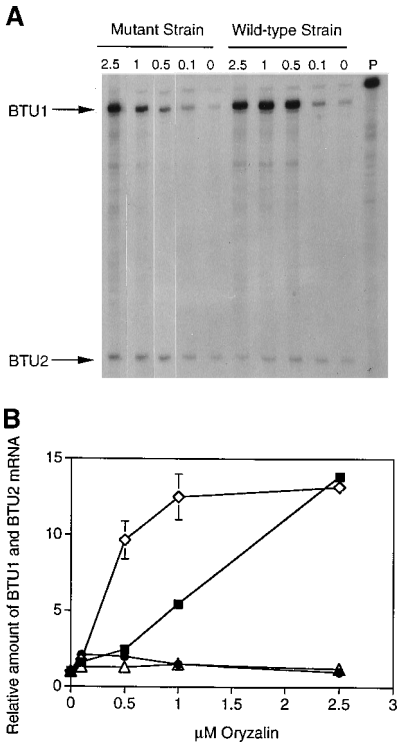


FIG. 6. The induction of *BTU1* mRNA is less sensitive to oryzalin treatment in oryzalin-resistant VB1 cells than in control cells. Starved *T. thermophila* cells were treated for 2 h with oryzalin at different concentrations (micromolar) as indicated above each lane. The abundance of β-tubulin mRNA was determined by an RNase protection assay. The bands shown in panel A were cut out and counted in a liquid scintillation counter, and the induction relative to zero time was plotted (B). ■, *BTU1* mRNA in VB1; ◇, *BTU1* mRNA in control cells; ●, *BTU2* mRNA in VB1; △, *BTU2* mRNA in control cells. Note that both the VB1 strain, containing the mutant *BTU1* gene, and the control strain, containing the wild-type gene, were cotransformed with the pH4T2 vector conferring neomycin resistance. Values are means and standard deviations from two experiments. Missing error bars are completely contained within the datum points.

and was not markedly induced in either transformed cell line. These results strongly suggest that the signal mechanism by which oryzalin treatment acts to increase expression of the *BTU1* gene involves the microtubule cytoskeleton.

**Taxol-hypersensitive VB1 cells show increased taxol sensitivity of *BTU1* mRNA induction.** VB1 cells show increased sensitivity to taxol. Taxol treatment of *T. thermophila* also induces the accumulation of tubulin mRNA (45). If microtubule drugs are inducing tubulin gene expression by a mechanism that involves the microtubule system, accumulation of *BTU1* mRNA in VB1 cells should occur at taxol concentrations lower than those at which accumulation occurs in control cells. As seen in Fig. 7, lower concentrations of taxol are required to induce *BTU1* mRNA accumulation in VB1 cells than are required to induce accumulation in cells transformed with pH4T2 alone. Note that in this experiment and in the one showing altered sensitivity of transformed cells to oryzalin (Fig. 6) we used the accumulation of tubulin mRNA as the indicator of altered drug sensitivity rather than run-on transcription, because of the difficulty in performing multiple nucleus isolations and obtaining quantitative drug dose-dependent run-on transcription data. Since in *T. thermophila* cells microtubule drugs appear to induce tubulin mRNA accumulation solely by changing the rate of transcription with no effect on mRNA stability (45), it seems very likely that in these transformed

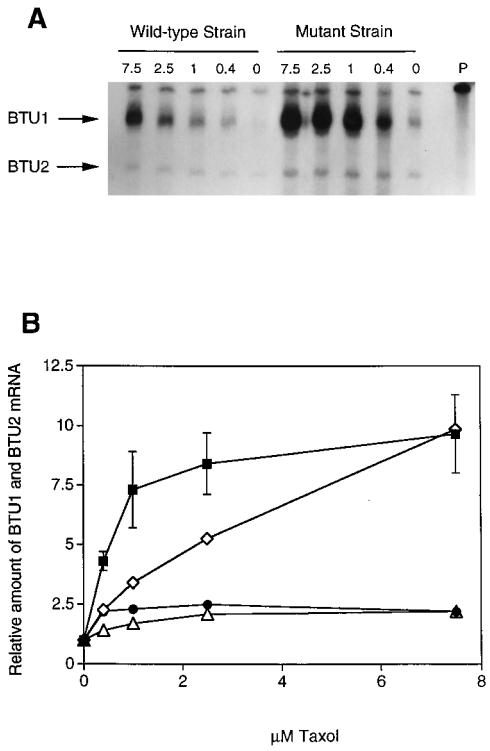


FIG. 7. The induction of *BTU1* mRNA is more sensitive to taxol treatment in taxol-hypersensitive VB1 cells than in control cells. Starved cells were treated with taxol for 2 h at different concentrations (micromolar) as indicated above each lane. The abundance of β-tubulin mRNA was determined by an RNase protection assay. The bands shown in panel A were cut out and counted in a liquid scintillation counter, and the induction relative to zero time was plotted (B). ■, *BTU1* mRNA in VB1; ◇, *BTU1* mRNA in control cells; ●, *BTU2* mRNA in VB1; △, *BTU2* mRNA in control cells. Note that both the VB1 strain, containing the mutant *BTU1* gene, and the control strain, containing the wild-type gene, were cotransformed with the pH4T2 vector conferring neomycin resistance. Values are means and standard deviations from two experiments. Missing error bars are completely contained within the datum points.

cells, the level of tubulin mRNA is a direct reflection of tubulin gene transcription.

**Drug-induced expression of *BTU1* parallels altered drug sensitivities in an α-tubulin mutant.** Strain E5 is a nitrosoguanidine-induced mutant strain that has a single amino acid replacement (Ala-65 to Thr-65) encoded in the single α-tubulin gene of *T. thermophila* (16). This mutation also confers resistance to oryzalin and hypersensitivity to taxol. When E5 or AG1 cells are treated with low doses of oryzalin (Fig. 8A) or taxol (Fig. 8B) and their RNA is isolated and analyzed by RNase protection, the inducibility of *BTU1* mRNA again parallels the altered sensitivities of cells to these drugs. The levels of *BTU2* mRNA remain essentially unchanged (data not shown). Thus, when the sensitivity of microtubules to drugs is altered by mutations specific to either the α-tubulin gene or the β-tubulin gene, drug-induced expression of *BTU1* is specifically altered in a parallel fashion. These results argue strongly that microtubules themselves or a microtubule-dependent process can initiate a signal that results in gene-specific transcription in *T. thermophila.*

## DISCUSSION

Two important observations emerge from the studies described here. First, it is clear that *T. thermophila* can distinguish between events that perturb ciliary microtubules and those that perturb cytoplasmic cytoskeletal microtubules and that it can respond with two different patterns of tubulin gene expression. When cilia are removed, transcription from all three tubulin genes (one for α-tubulin and two for β-tubulin) is induced and mRNA from each accumulates. When cells are treated with drugs that have been shown to perturb the cytoplasmic microtubule system without having any obvious effect on cilia (45), the α-tubulin gene and only one of the two β-tubulin genes are induced. To our knowledge, there is no previous example of tubulin gene-specific responses to different microtubule-containing organelles. Second, it seems clear that the microtubule drugs induce tubulin gene expression by a mechanism that involves the microtubules themselves, since tubulin mRNA accumulation in both β-tubulin and α-tubulin mutants having altered sensitivity to those drugs reflects that altered sensitivity. To our knowledge, this is the first demonstration that elements of the microtubule cytoskeleton play a role in the regulation of transcription of specific genes.

Because cilia are such unique structures, it is not difficult to conceive mechanisms by which *T. thermophila* can distinguish between ciliary and cytoplasmic microtubule elements. For example, it has been suggested that the cilia of the related protozoan *Paramecium caudatum* contain all of the voltage-sensitive calcium channels of the cell (31a). Cilia also contain unique dyneins (36) and kinesin (7), and those of *Tetrahymena* cells have been reported to contain a unique $Ca^{2+}$/calmodulin-activated tubulin kinase (25). Thus, one can envision the existence of cilium-specific signaling systems that stimulate the transcription of tubulin genes and other genes required for biogenesis of cilia. Rapid formation of cilia in other systems also is accompanied by induction of tubulin gene transcription (for reviews see references 33 and 45). It should be emphasized, however, that there is no evidence in any of these systems that transcription is regulated by microtubule (or other cytoskeletal) elements themselves. The existence of methods to create and identify cilium regeneration mutants (37, 38), along with recently developed methods for mass transformation of *T. thermophila* cells (18, 19), will, it is hoped, lead to the wherewithal to clone genes involved in this ciliary signaling system by complementation of mutant function.

It is more difficult to conceive mechanisms by which cytoplasmic microtubule elements are able to affect tubulin gene transcription in a gene-specific way. While it seems clear that the tubulin cytoskeleton plays such a role in *Tetrahymena* cells, speculations about the mechanisms involved must deal with the seemingly paradoxical observation that drugs that perturb

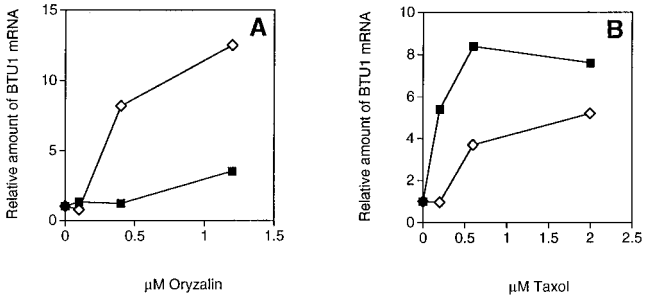


FIG. 8. The induction of *BTU1* mRNA is less sensitive to oryzalin and more sensitive to taxol in oryzalin-resistant, taxol-sensitive E5 cells than in the wild-type transformant AG1. *BTU1* message levels in E5 (■) and in AG1 (◇) were measured by RNase protection assays 2 h after treatment with either oryzalin (A) or taxol (B). Bands corresponding to the *BTU1* message were cut out and counted in a liquid scintillation counter. The induction relative to zero time was plotted for a single experiment with each drug.

the cytoskeletal microtubules by either depolymerizing or stabilizing microtubules induce the transcription of tubulin genes. It is worth noting that similar biological effects of depolymerizing drugs and taxol are not without precedent; these drugs have similar effects in disrupting the anterior localization of bicoid mRNA in *Drosophila melanogaster* oocytes (39). Also, recent studies indicate that substoichiometric levels of both taxol and the microtubule-depolymerizing drug vinblastine suppress microtubule dynamic instability in vitro (14) and that both drugs cause similar, concentration-dependent mitotic spindle abnormalities in HeLa cells at concentrations that produce little or no change in the amount of microtubule polymer (25a). As noted previously, one mechanism that could be operating in *T. thermophila* is that both types of drugs could effectively decrease the monomer pool, depolymerizing drugs by binding to tubulin monomer (or stimulating its degradation) and taxol by polymerizing it (45). *T. thermophila* would then be responding to decreased monomer levels by increasing tubulin gene transcription. This hypothesis can be tested by increasing the pool of free monomer in growing cells (in which the tubulin genes are actively transcribed) by microinjection and monitoring the effect on tubulin messages by in situ hybridization. Another possibility is that the cell has mechanisms for detecting the loss or abnormal function of polymerized cytoplasmic microtubules, possibly by monitoring some event associated with the dynamic equilibrium between tubulin monomer and microtubules. Depolymerizing drugs and taxol could affect this process similarly if, for example, both reduced some metabolic event associated with the dynamic instability of microtubules. For example, recent studies show that substoichiometric concentrations of taxol and vinblastine both suppress the rate of microtubule shortening in vitro (14). Given our finding that mutations which appear to affect microtubule stability in vivo show altered sensitivity of *BTU1* gene expression to antimicrotubule drugs, this hypothesis is particularly attractive. A third possibility, which could also explain the seemingly paradoxical ability of both depolymerizing drugs and taxol to disrupt localization of bicoid mRNA in *D. melanogaster* (39), is that taxol-polymerized microtubules fail to bind a microtubule-associated protein that is also released by microtubule depolymerization. In *D. melanogaster*, such a microtubule-associated protein could be involved (directly or indirectly) in binding to the bicoid message. In *T. thermophila*, such a microtubule-associated protein would (directly or indirectly) function to transduce a signal that specifically affects the transcription of the *BTU1* gene.

It should be noted that our studies do not indicate whether the role of microtubules in transducing a signal to the nucleus is direct (e.g., involving molecules directly associated with microtubules) or is an indirect consequence of altered microtubule function in some other cellular process (e.g., cell shape or organelle transport). In either case, the mechanism must involve a microtubule-mediated signaling event that is similarly affected by depolymerizing and polymerizing drugs. Whatever the mechanism, the studies described here define its existence and some of its properties. It should now be possible to identify factors, presumably proteins, that interact specifically with the α-tubulin gene and the *BTU1* gene but not the *BTU2* gene and whose activity in macronuclei of starved *Tetrahymena* cells increases as a result of treatment with either oryzalin or taxol. Once these factors are identified, it should be possible to define and analyze other components by which a signal is transmitted from the microtubule cytoskeleton to the α-tubulin and *BTU1* tubulin genes. Interestingly, the α-tubulin gene and *BTU1* have the same 7-nucleotide sequence (ATTTAAG) about 120 bp upstream of the initiator ATG that is embedded in a larger region in which all three genes are similar (21, 33). The *BTU2* sequence in this region (AAATACA) is identical to the sequences of the other two genes at only three positions. Soares et al. (44) also noted that this arrangement was found in the single α-tubulin gene and one of the two β-tubulin genes in *T. pyriformis*, and the heptanucleotide sequence is also completely conserved in the α-tubulin gene upstream regions of two other species, *Tetrahymena malaccensis* and *Tetrahymena canadensis* (32a). Such a region upstream of the tubulin genes could function in both the coordinate regulation of all three genes during growth and cilium regeneration and to distinguish *BTU2* expression from that of *BTU1* and the α-tubulin gene when cytoplasmic microtubules are perturbed.

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