THE EFFECT OF TRANSCRIPTION INHIBITORS ON UPTAKE OF URACIL AND URIDINE BY SCHIZOSACCHAROMYCES POMBE

by

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The effect of Tetrahymena derived inhibitor peptide(s) and Actinomycin D on uptake and incorporation of uracil and uridine in Schizosaccharomyces has been studied. It was found that the two transcription inhibitors initially caused some stimulation of uptake of both RNA precursors. With time Tetrahymena peptide reduced the rate of uridine uptake and incorporation dramatically, whereas the effect on uracil uptake and incorporation was much less pronounced. In the presence of Actinomycin D uptake of both precursors was stimulated even at a time when a reduced amount of incorporated label was observed.

1. INTRODUCTION

It has previously been described that (a) small peptide(s) secreted by the ciliated protozoan Tetrahymena pyriformis reduce the rate of RNA synthesis in the fission yeast Schizosaccharomyces pombe (1, 4, 5). This was measured chemically as well as by use of radioactive RNA precursors. However, the observed effect of the Tetrahymena peptide differed when the rate of RNA synthesis was measured by use of different labelled precursors. Thus, in exponentially mul-

tiplying cultures, the rate of incorporation of ¹⁴C-uracil and ³²P-phosphate into acid-insoluble material was reduced by 15-20%, whereas the rate of ¹⁴C-uridine incorporation was reduced by 50-70% under the same experimental conditions (4).

Like Tetrahymena peptide(s) the peptide antibiotic Actinomycin D reduced RNA synthesis in Schizosaccharomyces (5, 7). It was reported by MITCHISON and coworkers that uridine incor-

poration was affected differently from the other RNA precursors used, and they suggested that Actinomycin D not only affected transcription but also had a special effect on uridine uptake and/or incorporation (7). On this background we suggested that the different degree of inhibition of uracil and uridine incorporation by *Tetrahymena* peptide could be due to a special effect of this inhibitor on the uptake of uridine (4).

In the present paper we have investigated the effect of *Tetrahymena* peptide and Actinomycin D on uracil and uridine uptake by exponentially multiplying *Schizosaccharomyces pombe* cells. It is shown that both peptides affect the uptake of RNA precursors and it is suggested that this is a consequence of secondary, pool mediated regulations of the uptake following a reduced rate of transcription, rather than a direct interaction of the peptides with transport mechanisms.

2. MATERIALS AND METHODS

Cultures of the fission yeast *Schizosaccharomyces pombe*, strain 972 h⁻ (haploid wild type) were grown in complex nutrient broth, YEG, composed of 0.5% yeast extract (Difco) and 3% glucose. Except when otherwise indicated, uptake of radioactive uridine or uracil was determined in the following way, essentially according to the principles in MITCHISON and GROSS (6) and CUMMINS and MITCHISON (3):

Cultures were grown to a cell density of about 30×10^6 /ml (about 2 cell generations prior to entry into stationary phase) and divided in parallel portions of 20.0 ml. Partially purified *Tetrahymena* peptide (40 µl/ml) or Actinomycin D (Calbiochem, 20 µg/ml) was added at the appropriate time points. *Tetrahymena* peptide was prepared as described by ANDERSEN et al. (2) and partially purified on a G25 Sephadex column (Pharmacia) in order to remove low molecular weight substances.

Just prior to each uptake experiment the cultures were transferred to small beakers and stirred using a magnetic stirrer. At time zero radioactivity (0.13 µCi/ml) of ¹⁴C-uridine or ¹⁴C-uracil (Amersham, spec.act. 58 mCi/mmol in both cases) was added and samples were drawn alternately with a 1.0 ml automatic pipette for the determination of total radioactivity taken up

and the amount incorporated into acid-insoluble material, respectively. Incorporation was stopped by pipetting the samples into vials containing 1 ml ice cold 20 mM-uridine or 5 mM-uracil for the determination of total uptake and the same concentrations of uridine and uracil in 1 ml 10% TCA (trichloroacetic acid) for the determination of acid-insoluble counts. Each series of sampling lasted less than 2 min. Thus, changes in the temperature and, consequently, uptake rates could be ignored. After the termination of the experiment the samples were filtered and washed with the appropriate washing solutions: 10 mM-uridine, 2.5 mM-uracil or the same containing 5% TCA. The amount of uracil or uridine in the YEG medium was estimated to be less than 0.1 mM as determined by isotope dilution. Control experiments revealed that only insignificant amounts of radioactive material was lost from the cells during the washes when the label was not precipitated with TCA. The samples were dried and then counted in a Beckmann Liquid Scintillation Counter model LS 200.

Prior to addition of radioactivity, 2×1.0 ml of the experimental culture was removed and fixed with 1.0 ml 4% formaldehyde. Cell numbers were obtained from these samples after counting in a hemocytometer.

3. RESULTS

Figures 1 and 2 show the uptake and incorporation of ¹⁴C-labelled uracil and uridine, respectively, in cultures of *Schizosaccharomyces* which were treated with *Tetrahymena* peptide for 2 hours prior to addition of radioactive label. It is seen that in both cases total uptake and incorporation into acid-insoluble material are reduced by the peptide though more in the case of uridine (Figure 2A and B) than in the case of uracil (Figure 1A and B).

The content of labelled uracil or uridine in the acid-soluble pool can be calculated by subtraction of the values in Figures 1B and 2B (acid-insoluble counts) from the equivalent values in Figures 1A and 2A (total counts). The results are shown in Figures 1C and 2C, respectively. It is seen that the pool of both the control and the treated cultures reaches a steady state level about 20 minutes after the addition of the labelled compounds. The amount of radioactivity in the

Figure 1. The effect of Tetrahymena peptide on uptake and incorporation of ^{14}C -uracil in *Schizosaccharomyces*.

A: total amount of label taken up. B: label incorporated in the acid-insoluble material. C: label present in the cellular pool, calculated by subtraction of the values in B from the equivalent values in A. ^{14}C -uracil was added at time zero. Tetrahymena peptide was added 2 hours prior to the experiment. $\circ$: controls, $\bullet$: cells treated with Tetrahymena peptide.

Figure 2. The effect of Tetrahymena peptide on uptake and incorporation of ^{14}C -uridine in *Schizosaccharomyces*. Symbols and text as in Figure 1.

uracil pool at steady state is reduced by Tetrahymena peptide to about 85% of the control value, whereas the amount of radioactive uridine in the pool of the treated cells is only 54% of the control value.

It may be seen from Figures 1 and 2 that the amount of radioactivity incorporated into acid-insoluble material during the first 2 minutes is

Figure 3. The effect of Tetrahymena peptide on the initial uptake of uridine (left set of frames) and uracil (right set of frames) at various time points after addition of the peptide.

Circles: the total amount of label taken up. Triangles: label incorporated in the acid-insoluble material. Controls: open symbols; treated cells: closed symbols. Radioactivity was added at time zero. Each frame represents one separate experiment on identical parallel cultures.

Table I.

The effect of Tetrahymena peptide and Actinomycin D on initial uptake^{a)} and incorporation^{a)} of uridine and uracil by *Schizosaccharomyces*.

Time after addition of peptide	0 min		10 min		2 hours	
	uptake	incorp.	uptake	incorp.	uptake	incorp.
Uridine	Tetrahymena peptide		1.2	1.0	0.9	1.0
	Actinomycin D		1.2	1.0	-	-
Uracil	Tetrahymena peptide		1.1	1.0	1.1	1.0
	Actinomycin D		1.2	1.0	-	-
					1.5	0.6
					0.9	1.0
					1.4	0.7

a) Values are calculated as relative to the equivalent controls from readings at 30 sec on the curves shown in Figures 3 and 4.

low relative to the total amount taken up. Therefore, the initial total uptake is probably a reasonably good measure of transport of uracil and uridine into the cells. In order to study the initial uptake of the RNA precursors, experiments were designed to allow rapid sampling within the first minute after addition of the radioactivity. The uptake experiments were carried out at various time points after addition of Tetrahymena peptide. The results are shown in Figure 3. Each

frame represents measurements on identical parallel cultures as described in section 2. The results show (upper set of frames) that immediately after addition of Tetrahymena peptide the total uptake of both uridine (left frame) and uracil (right frame) tend to be increased. No difference in the amount of incorporated label could be detected at this time point. After 10 minutes in the presence of Tetrahymena peptide (middle set of frames) the total uptake of uridine is reduced compared to the control, whereas the uracil uptake is only slightly affected. Finally, after 2 hours in Tetrahymena peptide (lower set of frames) uridine uptake is strongly reduced whereas uracil uptake is only slightly reduced.

Assuming that the total amount of radioactivity taken up within the first minute reflects actual transport rates of precursor into the cells, the effect of the Tetrahymena peptide on transport and incorporation may be calculated. The results of such calculations are shown in Table I. It is seen that immediately after addition of the peptide, uptake of both uracil and uridine seem to be stimulated. After 2 hours in the presence of the peptide inhibitor uridine uptake is more than halved compared with the control while uracil uptake is only slightly affected.

As previously demonstrated (5,7) the peptide antibiotic Actinomycin D reduces the rate of RNA synthesis in *Schizosaccharomyces*. In order to see if this inhibitor had a similar effect on the uptake of uridine and uracil as described above, experiments analogous to the ones depicted in Figure 3 were carried out with Actinomycin D. The results are shown in Figure 4.

Figure 4. The effect of Actinomycin D on the initial uptake of uridine (left set of frames) and uracil (right set of frames). Text and symbols as in Figure 3.

As shown (upper set of frames) the uptake of both uracil and uridine is initially somewhat increased (cf Table I). The uptake pattern at 10 minutes after addition of Actinomycin D resembles that shown for zero minutes (not shown). However, contrary to the effect of the *Tetrahymena* peptide inhibitor, 2 hours treatment with Actinomycin D strongly increases the uptake of both uracil and uridine (Figure 4, lower set of frames). It may be calculated from the uptake curves that the uptake is increased by a factor 1.4 (cf. Table I). In spite of the increase in total uptake, incorporation of both uracil and uridine into acid-insoluble material is reduced to about 66% of the control values indicating an actually reduced rate of transcription (Figure 4, lower set of frames and Table I).

4. DISCUSSION

The results presented above show that in the presence of *Tetrahymena* peptide(s) uridine uptake and incorporation eventually become much reduced, whereas uracil uptake and incorporation is much less affected under identical experimental conditions. Actinomycin D affects both precursors in the same way, i.e. for both of them uptake is increased whereas incorporation is reduced.

Schizosaccharomyces growing in a complex nutrient broth as used here will utilize exogenous uridine and uracil as well as endogenously synthesized RNA precursors. The observations described here may therefore reflect 1) a direct interaction of the inhibitor peptides with uptake mechanisms at the cell surface or with intracellular enzymes involved in precursor uptake, 2) the existence of regulatory mechanisms controlled by the actual rate of transcription, the effects on uptake thus being indirect. The fact that uridine uptake eventually becomes strongly reduced after addition of the *Tetrahymena* peptide(s) could indicate that this inhibitor in addition to the previously reported effects on transcription (1, 2, 4, 5) acts as a rather specific inhibitor of uridine transport. However, the slowly develop-

ing effect on uridine uptake, with only a slight inhibition 10 min after addition of *Tetrahymena* peptide(s), makes a direct inhibition of transport mechanisms very unlikely. We therefore, favour the interpretation that the results obtained with both *Tetrahymena* peptide(s) and Actinomycin D are consequences of pool-mediated feed-back regulations of uptake following a reduced rate of transcription. Such regulation has previously been suggested to exist in *Schizosaccharomyces* with respect to adenine uptake (3).

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