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$tRNA^{Phe}$ AND MODIFIED FLUORESCENT NUCLEOSIDES IN SEVERAL SPECIES OF FUNGI

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Abstract—Fluorescent compounds were demonstrated at the level of $tRNA^{Phe}$ and in the free state in several species of yeast and mushrooms. The results are consistent with their having a role in general reproduction, both vegetative and sexual.

INTRODUCTION

The first demonstration of a fluorescent Y nucleoside was in *Saccharomyces cerevisiae* $tRNA^{Phe}$ in 1967 [1]. Similar molecules were subsequently found in the $tRNA^{Phe}$s of rat liver [2], ox liver [3], wheat germ [4] and rabbit liver [5]. These nucleosides are adjacent to the 3′-end of the anticodon and are implicated in phenylalanine codon recognition [6]. The biological importance of this molecule led to a number of structural studies [7–15]. Recently, the structure of a Yt component of *Torulopsis utilis* $tRNA^{Phe}$ was elucidated [16]. A cytokinin-like activity has been attributed to the Y compound [17] and we have attempted to detect this compound with biological tests [18]. We previously demonstrated the Y compound in the free state as a monophosphate ester in a *Daucus carota* tissue culture [19] and subsequently studied the evolution of this compound both at the level of $tRNA^{Phe}$ and in the free state in cells during the development of a haploid strain of yeast [20]. The present report is an extension of this study to several species of yeasts and mushrooms.

RESULTS AND DISCUSSION

The contents of $tRNA^{Phe}$, as mg/100 g fr. wt, and the fluorescence emission wavelengths of fractions excited at 330 nm are shown in Table 1. Table 2 shows the results obtained with strains grown in the laboratory: quantities of $tRNA^{Phe}$ at various developmental stages and the quantities of Y-like compounds, expressed in arbitrary fluorescence units.

Modified nucleosides in fungi have been studied by a number of authors. The rapid development of the mycelium of mushrooms and their fruiting modalities are consistent with the belief that mushrooms have a system of regulation implying the activity of growth factors, possibly including the modified nucleosides. Auxins, gibberellins and cytokinins were for a long time believed to be simple metabolites, as a result of the considerable quantities detected. It is currently believed that these molecules are growth and differentiation factors in fungi, as they are in higher plants. Although auxins and gibberellins have been extensively studied [21], cytokinins have been investigated to a lesser extent. We are personally interested by a fluorescent nucleoside termed 'Y', isolated as a component of certain $tRNA^{Phe}$s.

The Y nucleoside or related molecules have until the present been demonstrated in fungi only in *S. cerevisiae* and *T. utilis* as an integral part of cytoplasmic $tRNA^{Phe}$s. The present results show that the majority of the species studied contain a $tRNA^{Phe}$ which includes a Y-like fluorescent nucleoside, as shown by the fluorescence spectra obtained. The emission maxima, however, are quite variable, in the range 430–460 nm, implicating structural differences which would be interesting to determine in the future. It is noteworthy that in mammals (ox, calf, rabbit) and for the $tRNA^{Phe}$ of the same tissue — the liver — the same 'peroxy Y' molecule was demonstrated [22]. In the present work, we observed in most cases variable quantities of a free fluorescent nucleoside in the cell. Previously demonstrated in *S. cerevisiae* [20], these compounds have not, to our knowledge, been reported elsewhere. Here again, it can be seen that the emission spectra present several differences, perhaps indicating that these structures are not identical.

It is also seen that the amounts of $tRNA^{Phe}$ vary as a function of the species and for a given physiological state. They are relatively small in adult fruiting bodies and subsequently vary with the physiological state of a given species. This had previously been reported for *S. cerevisiae* [20] grown in complex medium and in situations where reproduction was exclusively vegetative. In this case, $tRNA^{Phe}$ levels reached their maxima in the middle of the exponential growth phase. The free Y compound, which was present in large quantities during the lag phase of growth, subsequently disappeared rapidly from the cell. It was found in the culture medium in variable quantities depending on time, and ongoing studies suggest that culture conditions have an important influence on the quantities of this compound [23].

The quantity of $tRNA^{Phe}$ in *D. uninucleatus* increases between 24 and 48 hr and the fluorescence spectra confirm determinations performed with UV spectrophotometry. A free Y-like fluorescent compound was demonstrated in almost identical quantities at 24 and 48 hr. Vegetative multiplication in this filamentous organism precedes sexual reproduction and this is a possible explanation for

Table 1. The $tRNA^{Phe}$ content and emission wavelength of 'Y-like' components when excited at 330 nm in various fungal species

	$tRNA^{Phe}$ (mg/100 g fr. wt)	Emission wavelength nm	
		'Y-like' excised from $tRNA^{Phe}$	Free 'Y-like' component
Dipodascus uninucleatus Biggs	21.30	440	440
Candida utilis (Henneberg) Lodder & Kreger-van Rij	25.00	430	430
Saccharomyces cerevisiae Hansen	1.45	430	430
Acetabula leucomelas (Pers.) Sacc.	1.13	430–460	435
Morchella esculenta Pers. ex St Amans	2.97	425	425
Sarcosphaera eximia (Duricu et Leveillé) R. Maire	2.12	-	430
Terfezia leonis Tul.	1.86	—	430
Rhizopogon luteolus Fr.	1.90	460	430
Langermannia gigantea (Batsch. per Pers.) Rosk.	1.80	430	440
Cantharellus tubaeformis Bull. ex Fr.	2.34	—	435
Melanopus squamosus (Huds) Pat.	0.50	460	445
Psalliota bispora Lange	3.40	430	435
Coprinus comatus Fr. ex Fl. Dan.	1.47	430	430
Coprinus atramentarius (Bull. ex Fr.) Fr.	2.83	435	435
Inocybe geophylla Fr. ex Sow.	1.14	450	430
Tricholoma albo. brunneum ss. Rick	0.22	—	430
Russula torulosa Bres.	0.34	460	430
Ixocomus luteus Fr. ex L.	0.70	460	425

the increased levels of a $tRNA^{Phe}$ rich in fluorescent nucleoside. The free compound, arising totally or partially from $tRNA^{Phe}$ turnover, is utilized within the cell or secreted into the medium. *P. bispora* is the richest in $tRNA^{Phe}$ during stages 2 and 3 of the mycelium and subsequently decreases rapidly when basidiocarps develop. A free fluorescent compound, present in important quantities in stage 3 mycelia, subsequently decreases relatively rapidly when basidiocarp initiation begins. It thus appears that the mycelium which prepares a fruiting body is the site of considerable $tRNA^{Phe}$ synthesis and that this species is rich in a fluorescent compound. The same compound is liberated in the free state by a rapid turnover. When primordia appear, these two elements decrease rapidly and this disappearance accelerates as the basidiocarp approaches complete maturity. $tRNA^{Phe}$ itself appears to become depleted of this fluorescent compound as the basidiocarp matures. The increase observed in *D. uninucleatus* which is developing a fruiting body is consistent with the belief that $tRNA^{Phe}$ and the fluorescent compound it liberates play a role in the differentiation of the mushroom. These results are to be compared with those [24, 25] which showed that cytokinin-type regulators are maximally present in the *Lentinus tigrinus* mycelium and then decrease as basidiocarps are initiated; this decrease is accentuated as maturation continues. Growth and differentiation processes are related to the concentrations of these growth regulators. They most probably play a role in basidiospore formation, in relation to nucleic acids and protein synthesis which precede basidium development.

The present report describes the first isolation of Y-like fluorescent nucleosides from the basidiocarps of higher mushrooms. In previous work on the evolution of $tRNA^{Phe}$ and the Y nucleoside in a haploid strain of *S. cerevisiae*, we obtained data suggesting that these molecules play an important role during protein synthesis, involved especially in cell division processes. It appears that they are also involved in sexual reproduction phenomena. Current

Table 2. The $tRNA^{Phe}$ content in mg/100 g fr. wt and content of 'Y-like' components in terms of arbitrary fluorescence units in *Dipodascus uninucleatus*, *Saccharomyces cerevisiae* and *Psalliota bispora*

	$tRNA^{Phe}$	'Y-like' compounds excised from $tRNA^{Phe}$	Free 'Y-like' compounds
Dipodascus uninucleatus			
24 hr	21.30	11 250	168 000
48 hr	40.20	21 300	145 600
Saccharomyces cerevisiae			
24 hr	1.45	980	Unquantifiable
48 hr	0.59	710	Unquantifiable
Psalliota bispora			
Mycelium 1	19.30	15 600	Unquantifiable
Mycelium 2	43.80	27 100	284 700
Mycelium 3	38.00	32 500	96 000
Sample 1	12.60	2060	26 000
Sample 2	11.80	1700	15 000
Sample 3	9.70	300	5500
Sample 4	3.40	109	4800

data on the sporogenic role of these molecules do not enable us to make definite conclusions and it may be supposed that the production of Y compounds would be necessary but not essential for triggering sporulation. The process of sexual reproduction, like that of asexual reproduction, may be accompanied by the synthesis of modified nucleosides. The present results are consistent with the participation of these molecules in general reproduction phenomena.

EXPERIMENTAL

Material. The species of yeast and mushrooms utilized are shown in Table 1. *Psalliota bispora* was grown in a cellar. The mycelium was sampled at 3 stages: m1 = 8 days, m2 = 15 days, m3 = 21 days, and then 4 times on fruiting bodies from the young primordium until the open adult basidiocarp (lots 1–4). Two yeast strains were grown in the laboratory for 24 and 48 hr. *Saccharomyces cerevisiae* X 2180-1 B (α) (generous gift of Prof. Mortimer, Berkeley, CA), a haploid strain, was grown in YEPD medium (1% yeast extract, 2% peptone, 1.5% glucose), agitated and aerated at 28°. *Dipodascus uninucleatus* 190.37 (Baarn, Holland) was grown in PDA (potato, glucose, agar) medium. *Candida utilis* (Baarn, Holland) was grown in YEPD medium for 24 hr.

tRNA and free fluorescent nucleosides. tRNAs were extracted as described [26], purified on DEAE-Sephadex and fractionated on BD-cellulose [27]: after washing the column with NaCl, $tRNA^{Phe}$ was eluted with 10% EtOH [28]. Fluorescent Y-like nucleosides were obtained after mild hydrolysis [29]. Purification was with 2-D desending PC on Whatman 3MM paper. Solvent 1: n-BuOH–MeOH–H_2O–NH_4OH (60:20:20:1); solvent 2: MeOH–H_2O–HOAc–HCl (77:11:20:2). Compounds were eluted with 0.1 N HCl and identified by R_f values, UV absorption spectra and fluorescence spectra in NaPi buffer, pH 7.

The free fluorescent nucleotide fraction was eluted by 1–4 M HCO_2H from a Dowex 1 × 8 (200–400 mesh) column after precipitation with TCA. They were then purified and characterized as above. In all cases, results are compared to those obtained with *S. cerevisiae* for $tRNA^{Phe}$ and free Y nucleoside [19].

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