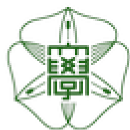
HOKKAIDO UNIVERSITY

Title	Level of ribosomal RNA in mycelia during conidiation in Aspergillus niger
Author(s)	WAKE, Kazutami
Citation	Journal of the Faculty of Science, Hokkaido University. Series 5, Botany, 12(1), 2-10
Issue Date	1980
Doc URL	http://hdl.handle.net/2115/26371
Type	bulletin (article)
File Information	12(1)_P1-10.pdf

Instructions for use

Hokkaido University Collection of Scholarly and Academic Papers : HUSCAP

Journ. Fac. Sci., Hokkaido Univ. Ser. V (Botany), **12** (1): 1–10, 1980.

Level of ribosomal RNA in mycelia during conidiation in *Aspergillus niger*

Kazutami WAKE

The cellular level of rRNA, expressed as rRNA/DNA (w/w), in *Aspergillus niger* mycelia has been determined under different culture conditions. In a standard surface culture, the level of rRNA increased markedly in pre-exponential growth period, decreased during early exponential growth period, and reached a low value. The level increased again with the conidiation. When the culture medium was replaced by sterile deionized water before conidiophore-forming period, the level increased at about the conidiating period without vegetative growth. In both a shaking culture and a nitrogen-excess culture which did not permit conidiation, a higher level of rRNA in early mycelial growth period decreased rapidly and reached a low value during the mycelial growth. When the nitrogen-excess medium was replaced by sterile deionized water in the stationary phase, the level increased again during the conidiation in non-growing mycelia. These results suggest that a cellular differentiation such as conidiation is accompanying with a new accumulation of ribosome independently of growth.

Radioactive isotopic labelling experiments in the basal culture showed that more than 50% of the preexisting hyphal rRNA was degraded during the early mycelial growth period (about 16.5 to 18.5 hr in duration) before conidiation.

In a previous communication, we reported qualitative differences among rapidly labelled RNA's synthesized during the conidication in *Aspergillus niger* under non-growing conditions (YOSHIOKA *et al.*, 1976). In this fungus, the growing mycelia contained RNA at a higher level and two peaks of total RNA content were found during early stage of culture (WAKE *et al.*, 1965). Furthermore, changes in fine structure of developing hyphal cells and conidiophore of *A. niger* were reported by TANAKA and YANAGITA (1963).

In these reports, information on the level of ribosomes in developmental stages of growth is not available so much. The regulation of the synthesis and accumulation of ribosomal RNA (rRNA) is one of the most important metabolic processes which ensure the control of cellular growth and development. Recently, ALBERGHINA et al. (1975) reported that the number of ribosome per genome increased dramatically by increasing the rate of growth

---

Abbreviations: PGM, potato-glucose-malt; rRNA, ribosomal RNA; tRNA, transfer RNA.

while the level of total protein remained almost unchanged and the level of tRNA increased only slightly in a *Neurospora.*

In the present paper the level and degradation of ribosomal RNA which characterizes *A. niger* cells in different conditions for development are reported.

Materials and Methods

Organism and growth conditions: *Aspergillus niger* A1015 was cultivated on the following media: Basal medium PGM was composed of 5% (w/v) glucose, 0.5% (w/v) NH_4NO_3 and a boiled extract prepared from 200 g potato and 50 g malt per one liter of tap water, and the initial pH was 4.3; N-excess medium was the same as PGM medium except the concentration of NH_4NO_3 (5%, w/v). For a non-shaking culture, flasks (300m*l*) containing 50 m*l* of growth medium were incubated with conida (20 μg conidia per m*l*, final concentration) at 30°C. For shaking culture, the flasks containing PGM medium were incubated at 30°C with a reciprocating shaker (135 strokes per min). For replacing culture, the PGM medium was replaced by 50 m*l* of sterile deionized water containing penicilin (15 μg/m*l*) and chloramphenicol (15 μg/m*l*), when a thin mycelial mat appeared in the early exponential growth period after 22 hr of 30°C incubation under a non-shaking condition. In the N-excess culture the medium was replaced by the sterile deionized water in the stationary phase of growth.

Labelling of mycelia with ^{3}H-uridine: To label the RNA, cultures were incubated for 60 min in a medium containing ^{3}H-uridine (5 μCi/m*l*) at a desired time. The radioactive mycelia were washed two times with sterilized solution of carrier uridine (40 μg/m*l*) at room temperature, then incubated with 50 m*l* of the same solution for 30 or 60 min at 30°C. In the N-excess culture, the washed mycelia were incubated with 50 m*l* of the N-excess medium containing cold uridine (40 μg/m*l*) at 30°C. The growth in this chase culture continued usually for 30 to 60 min at 30°C.

Extraction and fractionation of nucleic acids: Nucleic acids were prepared by a modification of the SDS-phenol method (Philipson, 1961). The frozen (−20°C) mycelial pads (about 10 g) were ground in chilled (2°C) mortars for 5 min in the presence of 20 m*l* of 0.1 M phosphate buffer (pH 7.2) containing 0.1 M NaCl, 2% sodium dodecylsulfate and 0.5% purified bentonite. An equal volume of 90% phenol was added to the homogenate, and after shaking 5 min in the cold, the suspension was centrifuged at 10,000 × *g* for 10 min. The aqueous layer was removed by pipetting and the phenol layer was re-extracted by adding an equal volume of 0.1 M phosphate buffer (pH 7.2) containing 0.1 M NaCl by the same procedures as

above. The combined aqueous layers were shaken with an equal volume of 90% phenol for 5 min. The mixture was centrifuged at $10{,}000 \times g$ for 5 min and the aqueous layer was removed by pipetting. The final aqueous layer was mixed with an equal volume of chloroform-isoamylalcohol (24 : 1, v/v) for 10 min. The upper layer separated on standing was centrifuged at $3{,}000 \times g$ for 5 min. The supernatant was dialyzed three times by 2000 m*l* of saline citrate buffer (0.15 M NaCl plus 0.015 M trisodium citrate, pH 7.0). All the above operations were done in a cold room at about 4°C. The nucleic acids were fractionated with MAK column chromatography (MANDELL and HERSHEY, 1960). Nucleic acids were applied to the column and eluted with a linear gradient of NaCl from 0.1 to 0.6 M. The optical density of each fraction (5 m*l*) was determined at 260 nm with a Hitachi spectrophotometer (Type 139). The following absorption coefficients at 260 nm have been used for the calculations: rRNA, 0.022/μg/m*l*; DNA, 0.020/μg/m*l*. The RNA/DNA ratio was expressed as a weight basis.

For the determination of growth curve, the DNA fractions from mycelia in different stages of growth were obtained by the method of SCHNEIDER (1945) and its amount estimated by the method of KECK (1956).

Preparation of ^{3}H-labeled rRNA: Four m*l* of whole rRNA fraction was precipitated by the addition of 4 m*l* of ice-cold 20% (w/v) trichloroacetic acid containing 10% (v/v) acetone. Trichloroacetic acid-preciptated rRNA was collected on Whatman glass fiber filters (GF/C) and washed with about 20 m*l* of cold 5% trichloroacetic acid. After drying, the filters were placed in 5 m*l* of toluene-based scintillation fluid and the radioactivity was measured using a Beckman liquid scintillation spectrometor (Model CPM-100).

Isotopes and chemicals: ^{3}H-uridine (5.0 Ci/mmol) was obtained from the Radiochemical Centre Amersham. Uridine was purchased from Kohjin Co., Ltd. All other chemicals were of analytical grade.

Results

Levels of rRNA in the mycelia growing under different growth conditions: In a non-shaking culture with PGM medium, a thin mycelial mat was formed in the early exponential growth period after 15 hr of incubation. Conidiophore and conidia were formed after 24 and 34 hr incubation, respectively.

The level of rRNA, expressed as rRNA/DNA (w/w), in the mycelia during the growth is shown in Fig. 1, in which the growth is expressed as the amounts of DNA per flask. The cellular level of rRNA increased rapidly in pre-exponential growth period, decreased during early exponential

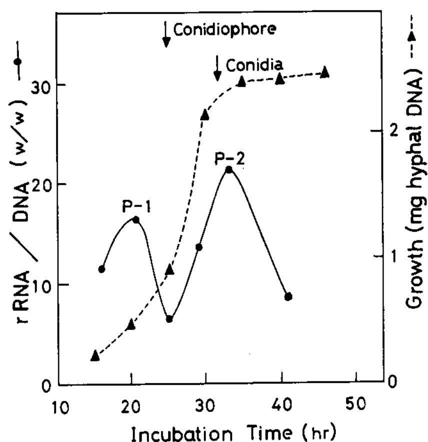

Fig. 1. Level of rRNA in mycelia of *A. niger* conidiating in basal midium. Experimental cultures were grown in 50 m*l* of PGM medium. The assay procedures of nucleic acids are described in Materials and Methods. The level of rRNA was calculated from the results by the MAK column chromatography. The ratio of RNA to DNA was expressed on a weight basis.

TABLE 1. Effect of concentration of NH_4NO_3 on growth of *A. niger*. The basal medium was modified with various concentrations of NH_4NO_3 and the extent of mycelial growth and conidiation at 30°C after 120 hr was compared. Whole mycelial pad containing conidia and the mycelial pad removed conidia by brushing and washing the pad surface were dried at 80°C and weighed. The difference between the two dry weights gives the determination of dried conidial matter

Concentration of NH_4NO_3	Conidia	Mycelia
%	mg dry wt./flask	
0.5	688	997
1.5	568	1017
2.5	330	1042
4.0	110	965
5.0	0	1032

growth period, and increased again during conidiophore-forming process.

By a convenient choice of the amount of NH_4NO_3 as a nitrogen source in PGM medium, it was possible to elicite a wide range of the rate of conidiation. The effect of concentration of NH_4NO_3 on conidiation and growth is shown in Table 1. Ammonium nitrate inhibited the conidiation at higher concentrations, but had little effect on growth.

As shown in Table 1, 2.5% NH_4NO_3 inhibited the conidiation about 50%, and 5% NH_4NO_3 inhibited completely. The effect of excess amount of NH_4NO_3 on the rRNA levels is shown in Fig. 2 and Fig. 3. The rRNA level reached the maximum (P-1) at pre-exponential growth period and decreased during mycelial growth, but as shown in Fig. 2 the second increase in rRNA level may be connected with conidiation.

The culture medium contained 5% NH_4NO_3 arrested the conidiation as stated above. However, when the culture medium was replaced by sterile deionized water in the stationary phase of growth, conidiation of non-growing mycelia proceeded normally. The rRNA level of non-conidiating mycelia decreased during the growth and reached a low level after about 70 hr of

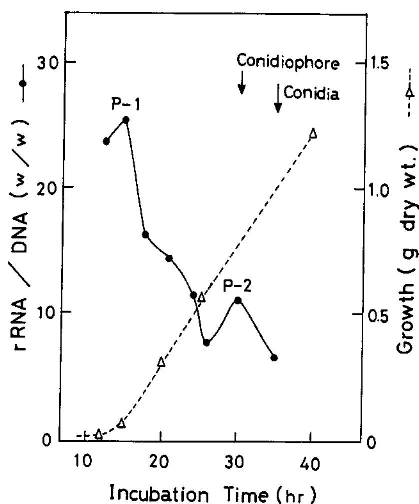

Fig. 2. Level of rRNA in mycelia of *A. niger* growing under partial repression of conidiation. Surface cultures were grown in 50 m*l* of PGM medium containing 2.5% NH_4NO_3. The level of rRNA was determined with analytical procedures as described in the legend to Fig. 1.

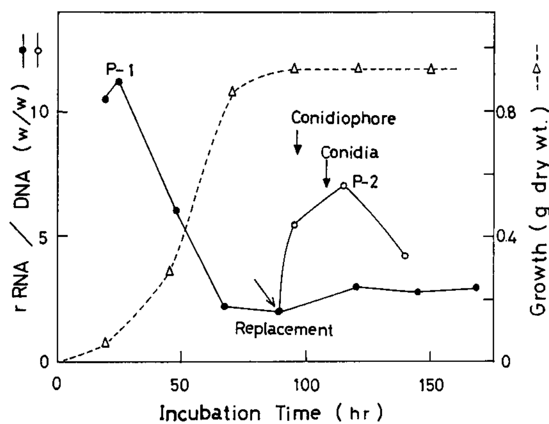

Fig. 3. Level of rRNA in mycelia of *A. niger* growing under repression and derepression of conidiation. Surface cultures were grown in 50 m*l* of PGM medium containing 5% NH_4NO_3. At 90 hr of growth, the culture medium was replaced by 50 m*l* of sterile deionized water. The level of rRNA was determined with analytical procedures as described in the legend to Fig. 1.

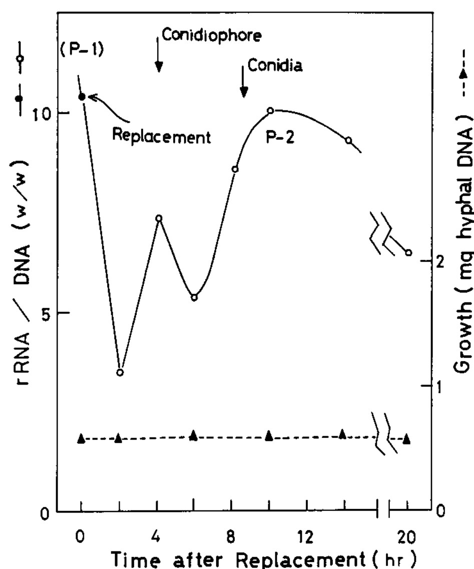

Fig. 4. Level of rRNA in mycelia of *A. niger* conidiating in non-growing medium. The culture medium was replaced by sterile deionized water after 22 hr culture as described in Materials and Methods. The level of rRNA was determined with analytical procedures as described in the legend to Fig. 1.

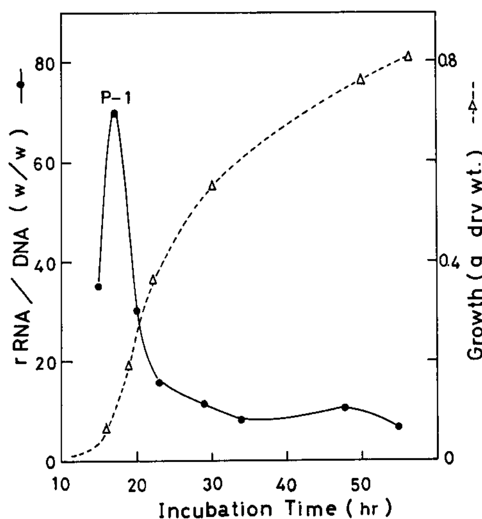

Fig. 5. Level of rRNA in mycelia of *A. niger* growing in shaking culture. The flasks containing 50 m*l* of PGM medium were incubated with a reciprocating shaker (135 strokes per min). At the time intervals indicated mycelia were collected by filtration. The level of rRNA was determined with analytical procedures as described in the legend to Fig. 1.

incubation, but after replacement the level in non-growing mycelia increased markedly during conidiation and reached the maximum (P-2) at about 20 hr after the replacement (Fig. 3).

The rapid increase in the level in non-growing and conidiating mycelia was also found in another replacing culture, in which PGM medium was replaced by sterile deionized water after 22 hr of incubation at 30°C. Under these conditions, the replaced medium arrested vegetative growth such as the increase of DNA content and dry weight of mycelia, but the conidiophore and conidia were formed at 4 and 9 hr after replacement, respectively. Fig. 4 shows the increase in the level of rRNA during the conidiation in the replacing culture. The level of rRNA reached a maximum (P-2) at 10 hr after the replacement.

In a shaking culture with PGM medium, conidiophore and conidia were not formed during the growth period. Fig. 5 shows that a higher peak (P-1) in the level of rRNA appeares in the early exponential growth period, then the level decreases rapidly during the mycelial growth and settles down after about 33 hr of incubation.

Our results show that a higher peak (P-1) in the level of rRNA appears in the early mycelial growth period under different growth conditions, and that the cellular level of rRNA increases again with the conidiation whether the mycelia are growing or not.

Ribosomal RNA breakdown: All the cultures gave similar results on a rapid decrease in the level of rRNA during early mycelial growth period. Measurements of rRNA breakdown were performed on mycelia labelled with ^{3}H-uridine for 60 min at different growth stages in the basal medium and

TABLE 2. Degradation of preexisting vegetative rRNA in conidiating mycelia. Cultures were grown in PGM medium. Labelling and assay procedures of rRNA are described in Materials and Methods. Relative specific activity of rRNA (cpm per μg rRNA) at 0 time of chase culture was expressed as 100.

^{3}H-Uridine incorporation	Chase (min)		
	0	30	60
period (hr)	relative specific activity		
15~16	100	105	59
17~18	100	43	66
20~21	100	83	77
23~24	100	89	81
29~30	100	101	124

the N-excess medium. The ribosomal RNA was labelled uniformly during a pulse of 60 min with ^{3}H-uridine (5 μCi/ml).

Labelled mycelia were transferred to a medium containing excess cold uridine (40 μg/ml). Table 2 shows the changes in specific activity of ^{3}H-rRNA during a chase with cold uridine. Previous experiments (Wake and Usami, 1970) showed that ^{3}H-uridine was linearly incorporated for about 60 min into rRNA from mycelia under various growth conditions. The results suggested that the rate of synthesis of rRNA was not changed during a chase culture. Therefore, it becomes apparent that changes in specific activity of rRNA in the chase is due to an effect on the rate of degradation of rRNA rather than on rRNA synthesis. After a chase with an excess of non-radioactive uridine, the specific activity (cpm/μg RNA) of prelabelled rRNA from mycelia in 18 hr culture fell precipitously and was reduced by half within about 30 min. The RNA degradation was responsible for the rapid decrease in level of rRNA in early mycelial growth period shown in Fig. 1. In a younger mycelia (15 hr culture), the specific activity decreased by about 40% after 60 min in a chase. Much less degradation of rRNA was observed in both 21- and 24-hr cultures, and the specific activity of preexisting rRNA in mycelia decreased by about 20% during a chase with cold uridine. In conidiating mycelia (30 hr culture), the preexisting ^{3}H-rRNA was conserved during a chase.

On the other hand, Table 3 shows that the most of the preexisting ^{3}H-rRNA from mycelia of early growth period in N-excess culture is conserved during the chase culture.

These results show that a degradation of preexisting vegetative ribosomes occurs markedly in a early mycelial growth period before conidiation, and the rapid degradation was not observed in the culture repressed conidiation.

Table 3. Degradation of preexisting vegetative rRNA in mycelia repressed conidiation. Cultures were grown in N-excess medium containing 5% NH_4NO_3. Labelling and assay procedures of rRNA are described in Materials and Methods. Relative specific activity of rRNA (cpm per μg rRNA) at 0 time of chase culture was expressed as 100.

^{3}H-Uridine incorporation	Chase (min)		
	0	30	60
period (hr)	relative specific activity		
24～25	100	80	88
29～30	100	89	86
39～40	100	101	96

Discussion

The accumulation of rRNA expressed as initial peak (P-1) was found in common in different cultures of *Aspergillus* cells, and this was followed by increase in rRNA level accompanying mycelial growth. ALBERGHINA *et al.* (1975) reported that the cellular level of rRNA was proportional to μ (doublings per hr) in *Neurospora* mycelia. It seems likely that the first increase of rRNA level of *Aspergillus* mycelia respond to increasing of the rate of growth in early mycelial growth stage. We have determined the degree of correlation between rRNA level and conidiation. Our results suggested that *Aspergillus* conidiating mycelia had a specific level of rRNA expressed as peak 2 (Figs. 1-4). Fig. 4 shows that the rRNA level increases dramatically during the conidiation while the level of total DNA in mycelia remains almost unchanged. We presume that a cellular differentiation such as conidiation may require a new accumulation of ribosome independently of growth.

In comparing the data of Tables 2 and 3 it suggests that the stability of the ribosmes accumulated in early vegetative growth period seems to be strongly correlated with conidiating process. In *Chamydomonus reinhardtii*, approximately 90% of the preexisting vegetative ribosomes were found to have degraded during the gametogenesis and much less degradation of tRNA was observed (SIERSMA *et al.*, 1971), and in yeast cells the breakdown of preexisting vegetative RNA and protein occurred much more extensively in sporulating than in non-sporulating cells (HOPPER *et al.*, 1974). These data suggest that such degradation and reutilization of rRNA and ribosomes may be involeved in key regulatory system in cellular differentiation of simple eukaryotic cells.

The experiments to determine the level of tRNA in *Aspergillus* mycelia are in progress. The level of tRNA changed slightly in early mycelial growth period, but much degradation of tRNA was observed in the stage of conidiation (WAKE, unpublished data).

We did not determine the relative rates of rRNA transcription under various growth conditions in this study. Because the nucleotide pool size in *Aspergillus* mycelia varies extensively during the growth (WAKE *et al.*, 1965), it is very important to find the labelling of the precursor pool that is relatively unaffected by the rate of protein synthesis or growth rate. The method of methyl labelling by SHULMAN *et al.* (1977) may be suitable for the determination of RNA synthesis in *Aspergillus* cells.

The author is grateful to Dr. S. USAMI for his interest in this work, and to Dr. S. SASAKI for his valuable comments on the manuscript.

References

ALBERGHINA, F. A. M., STURANI, E. and GOHLKE, J. R. 1975. Levels and rate of synthesis of ribosomal ribonucleic acid, transfer ribonucleic acid, and protein in *Neurospora crassa* in different steady states of growth. J. Biol. Chem. **250**: 4381-4388.

HOPPER, A. K., MAGEE, P. T., WELCH, S. K., FRIEDMAN, M. and HALL, B. D. 1974. Macromolecule synthesis and breakdown in relation to sporulation and meiosis in yeast. J. Bacteriol. **119**: 619-628.

KECK, K. 1956. An ultramicro technique for the determination of deoxypentose nucleic acid. Arch. Biochem. Biophys. **63**: 446-451.

MANDELL, J. D. and HERSHEY, A. D. 1960. A fractionating column for analysis of nucleic acids. Anal. Biochem. **1**: 66-77.

PHILIPSON, L. 1961. Chromatographic separation and characteristics of nucleic acids from *Hela* cells. J. Gen. Physiol. **44**: 899-909.

SCHNEIDER, W. 1945. Phosphorus compounds in animal tissues. I. Extraction and estimation of deoxypentose nucleic acid and pentose nucleic acid. J. Biol. Chem. **161**: 293-303.

SHULMAN, R. W., SRIPATI, C. E. and WARNER, J. R. 1977. Noncoordinated transcription in the absence of protein synthesis in yeast. J. Biol. Chem. **252**: 1344-1349.

SIERSMA, P. W. and CHIANG, K.-S. 1971. Conservation and degradation of cytoplasmic and chloroplast ribosomes in *Chlamydomonus reinhardtii*. J. Mol. Biol. **58**: 167-185.

TANAKA, K. and YANAGITA, T. 1963. Electron microscopy on ultrathin sections of *Aspergillus niger*. I. Fine structure of hyphal cells. J. Gen. Appl. Microbiol. **9**: 101-118.

———. 1963. Electron microscopy on ultrathin sections of *Aspergillus nigir*. II. Fine structure of conidia-bearing apparatus. J. Gen. Appl. Microbiol. **9**: 189-203.

WAKE, K. and USAMI, S. 1970. Proc. 35st. Ann. Meeting of Bot. Soc. Japan. p. 77. (in Japanese)

WAKE, K., YAMASHITA, T. and SASAKI, S. 1965. Studies on the physiological process during the growth and development of *Aspergillus niger*. J. Fac. Sci., Hokkaido Univ., Series 5. **8**: 211-219.

YOSHIOKA, N., WAKE, K. and USAMI, S. 1976. Rapidly labelled RNA from conidiating mycelia of *Aspergillus niger*. Plant and Cell Physiol. **17**: 1081-1084.