KOR. JOUR. MICROBIOL. March, 1988 p. 27-31 Vol. 26, No. 1

Biosynthesis of messenger RNA in *Aspergillus phoenicis* during their life cycle

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*Aspergillus phoenicis*의 생활사를 통한 mRNA의 생합성

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ABSTRACT: Biosynthesis and processing of cytoplasmic mRNA from heterogenous nuclear RNA (hn-RNA)in *Aspergillus phoenicis* were studied by ^{3}H-uridine labeling and synchronous culture techniques during their life cycle.

Incorporations of ^{3}H-uridine into hn-RNA and mRNA were most rapid in vesicle-phialide formation stage and diminished in hyphal growth stage. The processing of cytoplasmic mRNA from hn-RNA was proceeded more rapidly in hyphal growth and conidiophore formation stages than in conidia and vesicle-phialide formation stages. The specific radioactivities of hn-RNA and mRNA were very high in vesicle-phialide formation stage.

KEY WORDS □ *Aspergillus*, Synchronous culture, mRNA biosynthesis.

Incorporation of radioactive precursors into RNA continued at a high rate throughout the period of differentiation (Kim, 1971). In vegetative microorganisms, the differentiation was accompanied with the stable development of altered structure and function. Then, the synchronous culture was very important in the study of the biosynthetic activities at morphologically different structure of fungi. Smith *et al.*, (1971) have developed the synchronous initiation of conidiophore in *Asp. niger* with the method of submerged-liquid culture and Kim (1971) have it accomplished at the four distint morphogenetic structures in *Asp. niger* during their life cycle.

The evidences of that heterogenous RNA of nucleus was precursor of cytoplasmic mRNA have been demonstrated in several studies (Penman *et al.*, 1968; Lee *et al.*, 1971; Darnell *et al.*, 1973; Udem and Warner, 1972, etc). mRAN synthesis in eukaryotes is dominated by the very large, rapidly synthesized heterogenous nuclear RNA (Darnell, 1968). The processing of mRNA from hn-RNA in fungi have some similarity to that of mammalian cells (Darnell, 1968; Darnell *et al.*, 1973). It was postulated that RNA synthesis in eukaryotes was controlled by the interplay of at least two distinct fractions. One control the rate of the ribonucleotide polymerization process and the other, the number of enzymes molecules bound to the DNA templates (Gross and Pogo, 1974).

The purpose of the present study was that the biosynthesis and processing of mRNA at different stages during their life cycle of *Asp. phoenicis* were obtained using synchronous culture and ^{3}H-uridine labeling techniques.

MATERIALS AND METHODS

Organism

Aspergillus phoenicis K.U. 117 was used in this experiments and stock culture of this maintained on Czapek's agar media for 7 days 35°C. Spores were harvested with steriled cold water and collected through a sintered glass filter, after that, it

was washed three times by centrifugation for 10 min, each at 4,000rpm. After counting spores in a haemocytometer, the suspension was used as the inoculum for the synchronous culture.

Synchronous culture

This media has been designed and developed by Smith *et al.*, (1969 and 1971). (Table 1)

Glucose was autoclaved separately and added aseptically. Each flask was inoculated with 5×10^5 spores/m*l* at 35°C. The first medium replacement at 48 hours induced conidiophore, in MediumB for 32h. But, conidiophore initiation occured under continous oxygen limited conditions into 0.04 Lpm(liter/min) although vegetative growth was not restricted. After that, continuously conidiophores were elongated at 0.3Lpm of aeration rate. The second medium replacement at 80h. leads to vesicle-phialide production for 24h. Finally, conidia formation were induced from inoculum for liquid shaking culture. Growth and synchronous development of hypha, conidiophores and vesicle-phialide were obtained by means of a replacement fermentor culture technique, but conidia were formed in 50 m*l*. medium into 250 m*l* Erlenmeyer flasks.

Cell lysis and fractionation

The lysis and separation of mycelium into two fractions were done by the method of Greenberg and Penman (1966), Lee *et al.*, (1971). This procedures were shown in Figure 1. The mycelium of

Table 1. *Constituents of media*

Constituents	Unit	Medium A	Medium B	Medium C	Medium D
KH_2PO_4	g	1.0	1.0	1.0	13.0
K_2HPO_4	g	—	—	—	1.13
$MgSO_47H_2O$	g	0.25	0.25	0.25	0.25
$CuSO_47H_2O$	mg	0.234	0.234	0.234	0.234
$FeSO_47H_2O$	mg	6.32	6.32	6.32	6.32
$ZnSO_47H_2O$	mg	1.1	1.1	1.1	1.1
$MnCl_24H_2O$	mg	3.5	3.5	3.5	3.5
$CaCl_2$	mg	46.7	46.7	46.7	46.7
$(NH_4)_2SO_4$	g	3.96	0.66	1.98	—
$NaNO_3$	g	—	—	—	5.1
Glucose	g	20.0	10.0	—	20.0
Citric acid	g	—	—	12.6	—
Silicone	m*l*	—	1	1	—
D.W.	*l*	1	1	1	1
pH		2.3	4.6	4.6	5.5

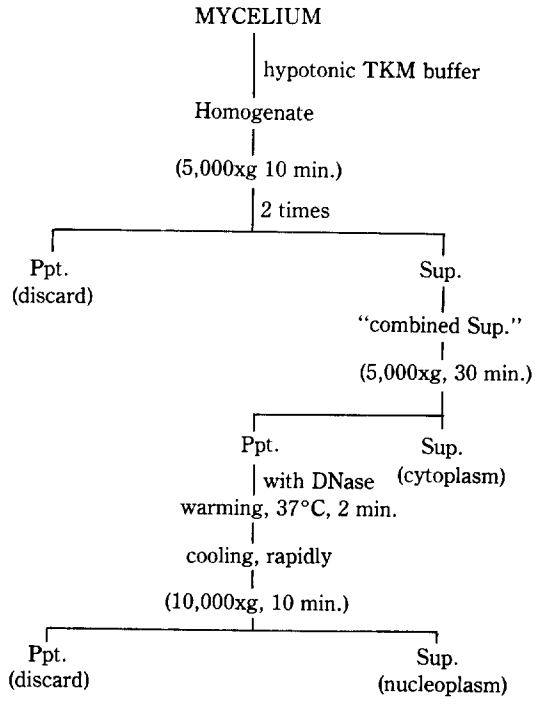

Fig. 1. *Subcellular fractionation from the mycelium of A. phoenicis.*

filtered in Whatman glass filters washed with ice-cold distilled water by 2-3 times. That was resuspended in 2 m*l* of hypotonic buffer allowed to swell for 10 min, and broken with stone pieces 5g for wet weight 300mg. The broken cells were sedimented by centrifugation at 5,000g for 10 min, twice. All of sup. was disrupted in 2.5 times of Triton lysing medium (50 mM Tris-HCl, pH 7.6, 1.30 mMKCl, 6.5 mM-mercaptoethanol, 0.13% Tritonx-100, 13% sucrose) and then centrifugated at 5,000g for 30min. The supernatant was yielded as the cytoplasmic fraction and the ppt. was added with DNase (5μg/m*l*), warming at 37°C for 2 min and rapidly cooled to 0°C. That of centrifugated at 10,000g for 10 min was yield the nucleous from supernatant. All of operations was carried out at 0°-4°C.

Cell labeling and polysome preparation

Biosynthesis and processing of cytoplasmic messenger RNA precursor during their life cycle of *Asp. phoenicis* were studied by using ^{3}H-uridine (29Ci/mole) labeling. The procedure was modified by the method of Boehlke and Friesen (1975). Labeling and growth were stopped by the additions of NaN_3, cycloheximide to 10M and 200 ug/m*l* final concentrations, respectively. That was swol-

len in hypotonic TKM buffer and polysome was isolated from each other fractions as follows (Lee and Yoon, 1978) The cells were ultracentrifuged at 41,000rpm for 3 hours, 4°C and the ppt. was yielded as polysome. If the labeled samples were small, that were added with $MgCl_2$ at final concentration of 30mM and released for 30 min at 0°C, and then centrifuged at 2,000 rpm. The ppt. was yielded as polysome.

RNA extract

All operations were carried out at 0°-4°C by the method of Lee *et al.*, (1971). The polysomes in 0.1M Tris-HCl buffer of pH 9.0 were diluted into 0.5% sodium dodecyl sulfate and shaken for 10 min with an equal volume of redistilled 75% aqueous phenol. The phases were separated by centrifugation at 2,000rpm for 10min. The residue after removal of the aqueous phase was re-extracted twice with phenol, and the RNA was precipitated overnight with 2.5 volumes of 95% ethanol and 0.1 volume of 1% NaCl at -20°C. The ppt was washed three times with 66% aqueous ethanol in 0.03% NaCl, dissolved in TKM (10mM Tris-HCl, 10mM KCl, 1mM MgCl) buffer of pH 9.0 and directly used in experiment. Of the upper procedures, rRNA was extracted in TKM buffer exchanged to pH 7.6. But, hn-RNA was extracted as follows (Lee and Yoon, 1978). Nucleoplasm of *A. phoenicis* was diluted into lysing medium and dissolved perfectly. Then, it was added with 0.1M sodium acetate (pH 5.2) of ice-cold and 0.5% SDS. This solution was shaken with an aqual volume of 75% aqueous phenol, dissolved for 10min at 0°-4°C and heating for 5min at 65°C, then rapidly cooled to 0°C. The phases were separated twice by centrifugation at 6,500rpm for 15 min. These residues were combined and extracted into hn-RNA as mRNA.

Radioactivity measurements

The measurements of radioactivities of mRNA and hn-RNA were determined by the method of Lee *et al.*, (1971). For the millipore binding measurements of hn-RNA and mRNA, samples were diluted into 10 volumes of ice-cold 10mM Tris (pH 7.6) -500mM KCl-1 mM MgCl. They were filtered through millipore filters (HA 0.45 um) previously soaked in the same salt solution after 10min from that and the filters were washed twice with 10m*l* of salt solution. After drying, they were counted in toluene scintillation mixture. But, the radioactivity of rRNA was measured of the method of Lee and Yoon (1978). The samples were precipitated with ice-cold 10% TCA and collected on glass fiber filters, washed with 0.1%, 10% TCA. It was dried, placed in toluene scintillation mixture and counted in liquid scintillation counter (Backman LS 100).

RESULTS AND DISCUSSION

Synchronous culture of *A. phoenicis* KU 117

Synchronous growth curve in *A. phoenicis* was obtained in four kinds of replacement medium during their life cycle. (Fig. 2) It exhibited that the spores germinated at 3 to 4 hours and hypha extended rapidly at 18 to 48 hours in Medium A. Continuously, the conidiophores formed till 80 hours in low-nitrogen medium (medium B) and the vesicle initiated at 6 hours after replacement with citrate ammonium medium (medium C). Finally, initiation of conidia occured in nitrogen glucose medium (medium D) at 26 hours after replacement. The growth rate during their life cylce of *A. phoenicis* was related about 50 hours than *A. niger* (Kim, 1974).

Incorporation of ^{3}H-uridine into RNAs

The rate of incorporation of ^{3}H-uridine into ribosomal RNA (rRNA) was obtained as the difference with that of heterogenous nuclear RNA (hn-RNA) and messenger RNA (m RNA) during their life cycle of *A. phoenicis* (Fig. 3). It showed that two biosynthesis of hn-RNA and mRNA in *A. phoenicis* were not absolutely necessary for hypha and conidiophore formation, the other hand, that of rRNA occured from the initiation of hypha, continuously. Also, the majority of the nucleotide incorporated by ^{3}H-uridine was occured in vesicle-

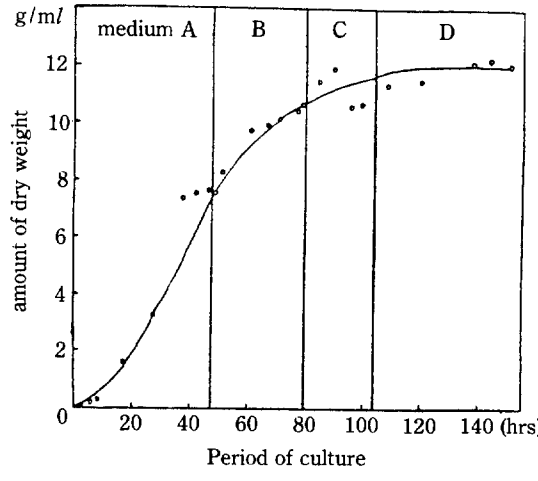

Fig. 2. *Synchronous growth curve of A. pheonicis.* The vegetative mycelial growth was conducted in "Medium A", conidiophore in "Medium B", vesicle & phialide in "Medium C" and sporulation occured in Medium D".

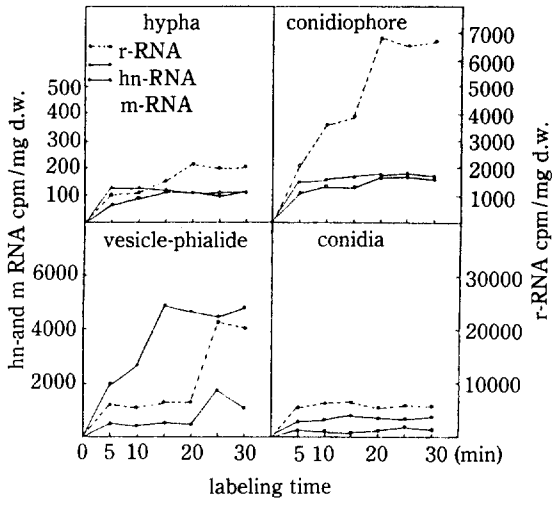

Fig. 3. *Labeling characters of RNAs treated with ^{3}H-uridine during their life cycle of A. phoenicis.*

phialide formation stage of *A. phoenicis.* This experments was very similar with the result of RNAs biosynthesis in *Fibroblasts* (Mauck and Green, 1973).

Specific radioactivities of RNAs

The changes in the specific radioactivities of RNAs incorporated with ^{3}H-uridine in vesicle-phialide formation state of *A. phoenicis* was exhibited in Fig. 4. During short periods (30 min) of ra-

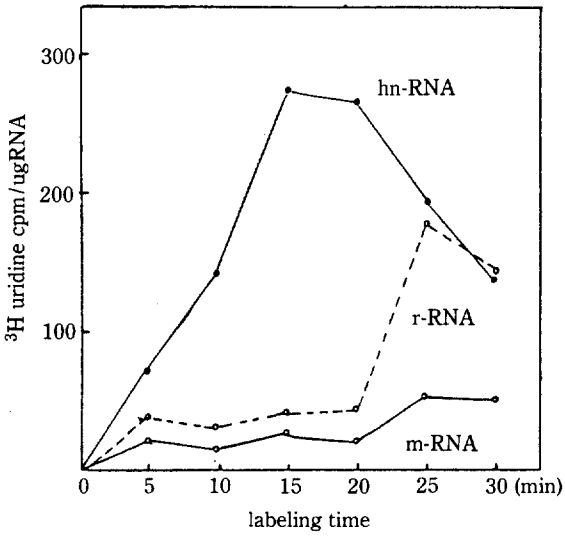

Fig. 4. *The specific radioactivity of various RNAs in A. phoenicis treated with ^{3}H-uridine at vesicle & phialide formation stage.*
The fungi were harvested and treated with ^{3}H-uridine at 100 hours after inoculation.

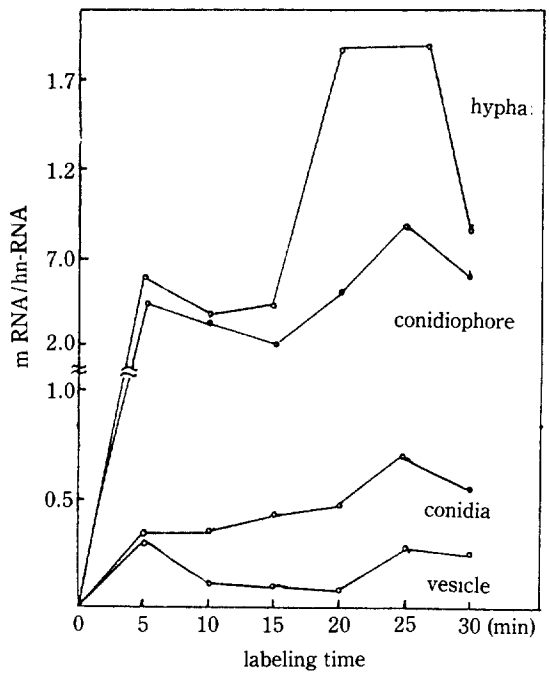

Fig. 5. *Changes in the ratio of specific radioactive mRNA per specific radioactive hn-RNA of A. phoenicis treated with ^{3}H-uridine during their life cycle.*
The fungi were harvested and treated with ^{3}H-uridine at 10 hours (hypha), 72 hours (conidiophore), 100 hours (vesicle-phialide) and 137 hours (conidia) after inoculation, respectively.

dioactive isotope incorporation, the specific radioactivity of hn-RNA reached at the maximum in a 15-minute, indicating that it turned over rapidly in the nucleus. Also, this suggested that it was necessary an approximately 10-minute lag before the appearance of mRNA on polyribosomes in cytoplasm. This result was compared with delaying for 15-minute in Hela cells (Penman *et al.*, 1968).

Processing of mRNA from hn-RNA

The ratio of specific ratioactive mRNA to that of hn-RNA during their life cycle of *A. phoenics* was showned in Figure 5. The biosynthesis of mature mRNA required well-controlled cytoplasmic and nuclear interactions, since all the processing steps took place in the nucleus. Although, the rate of turn over of mRNA different to that of hn-RNA, the rate of processing of mRNA from hn-RNA in *A. phoenicis* was very high in hypha and conidiophore formation stages, whereas, it was very low in vesicle-phialide and conidia formation stages during their life cycle. Further studies of the mutants which effect mRNA splicing and proces-

sing should help the efforts to resolve the steps involved in mRNA biosynthesis, however, it is clear that generation of mature mRNA require 5'capping enzymes, 3'-poly(A) polymerase and mRNA splicing enzymes. (D. Apirion, 1984).

적 요

A. phoenicis K.U. 117의 생활사를 통한 mRNA의 **생합성** 및 processing 과정을 동조배양법과 ^{3}H-uridine을 추적자로 사용하여 밝히고자 하였다. ^{3}H-uridine의 RNA로의 전환은 hn-RNA, mRNA 및 rRNA 등 모든 RNA가 경자형성기에서 가장 빨랐고, 이때의 각 RNA의 비방사능은 hn-RNA, rRNA, mRNA 순으로 높았다.

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(Received Oct. 19, 1987)