EARLY MATING INTERACTIONS IN SCHIZOPHYLLUM COMMUNE

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This is to certify that the

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Richard W. Bingham
Major professor

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ABSTRACT

EARLY MATING INTERACTIONS IN SCHIZOPHYLLUM COMMUNE

By

John Vincent Leary

The objective of this research was to approach the study of the earliest mating interactions in Schizophyllum commune Fr. on two levels, the biological phenomenon of nuclear exchange and the molecular changes associated with mating.

On the biological level, experiments were conducted to determine the kinetics of the initial nuclear exchange between homokaryons in the compatible and non-compatible mating combinations. Techniques were developed for mating mycelial fragments in liquid culture. Evidence for nuclear exchange was based on analysis of fragments derived from the developing heterokaryon which possessed nuclei from all 4 types of matings. The kinetics of nuclear exchange were relatively independent of the method used. In all 4 types of matings, compatible, common-A, common-B, and common-AB, the percentage of fragments possessing both types of nuclei 12-24 hr after mating is nearly equal. After this time, significant differences become evident in the kinetics of the compatible vs. the 3 non-compatible matings. Differences are also evident in the common-A vs. the common-B and common-AB matings. The percentage of mycelial fragments possessing both nuclear types throughout the 96 hr test period is similar for both the common-B and common-AB matings. The kinetics of nuclear exchange were independent of the particular mating-type alleles and nutritional markers used. Nuclear exchange occurred more rapidly and synchronously in minimal medium than in complete medium. This difference in mating efficiency is not due to growth

differences in the two media. In the majority of fragments analyzed in a compatible mating at 0 hr and 48 hr after mating, the number of cells/fragment was fewer than 5, indicating that the interactions observed were closer to a cell-to-cell basis than was possible before.

These studies indicated that the earliest mating interactions, i.e. hyphal anastomosis and nuclear exchange, are independent of the mating-type factors but that subsequent events are determined by these genes. Also, support is given to the hypothesis that the incompatibility reaction is not constitutive but induced.

These studies formed a basis for the design of experiments to investigate the incompatibility reaction on the molecular level. To facilitate such experiments, it was necessary to develop methods for isolating large amounts of ribosomes and polysomes from Schizophyllum mycelia. It was shown that functional monosomes and polysomes could be readily isolated from mycelia and spores which were lyophilized and then ground dry. The monosomes were shown to have an approximate sedimentation value of 80S. Polysomes consisting of 2, 3, and 4 ribosomes were consistently present. The polysomes were stable at low magnesium concentration and were sensitive to ribonuclease treatment. The ribosomes were functional in a cell-free amino acid incorporation system. This incorporation occurred in the absence of exogenous messenger RNA, indicating that the ribosome-mRNA complex was stable after lyophilization and grinding.

The approach to the study of the molecular basis of the incompatibility reaction was to investigate the possibility that cytoplasmic exchange occurred at the same time as nuclear exchange. These experiments were conducted by differentially labelling the homokaryotic mating partners with

deuterium and a radioactive amino acid. Appearance of radioactive label on the heavy ribosomes was considered evidence for cytoplasmic exchange. The ribosomes isolated from such mycelia could be separated by CsCl equilibrium density-gradient centrifugation. In matings between homokaryon grown in D_2O and a homokaryon labelled with a radioactive tracer, evidence was obtained that exchange of cytoplasm had occurred since both the heavy and light ribosomes were radioactive. The heavy ribosomes were greatly reduced in number after 24 hr of mating in H_2O-medium. Considerable synthesis and rapid turnover of ribosomes was indicated. This was confirmed by analysis of matings after 12 hr and 24 hr of mating in H_2O-medium. After 12 hr of mating, the heavy ribosomes were decreased in concentration and ribosomes of hybrid density were present. After an additional 12 hr of mating the hybrid ribosomes were almost gone and an increase in quantity of "light" ribosomes was evident. Additional evidence for cytoplasmic exchange was that the "light" ribosomes increased in density progressively after 12 hr and 24 hr of mating. This was interpreted to mean that the D_2O in the cytoplasm of the D_2O-labelled homokaryon was made available to the mating partner via exchange of cytoplasms and that ribosome synthesis in the developing dikaryon occurred in a common cytoplasm which increased in D_2O content as cytoplasmic exchange increased.

EARLY MATING INTERACTIONS IN *SCHIZOPHYLLUM COMMUNE*

by

John Vincent Leary

A THESIS

Submitted to
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To

My Wife, Barbara

and

My Three Sons

John, Patrick and Timothy

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INTRODUCTION

In the Basidiomycete Schizophyllum commune Fr., sexual reproduction and the formation of the fruiting body must be preceded by the formation of the dikaryon.

The process of dikaryosis has been shown to be a coordinated series of events in which the vegetative homokaryon with one nucleus per cell is transformed into the dikaryon. The dikaryon is unique in that it is capable of indefinite vegetative growth in a state where each cell possesses two genetically distinct nuclei.

The events leading to formation of the dikaryon include hyphal anastomosis, reciprocal nuclear exchange, extensive nuclear migration, and conjugate nuclear division with concomitant clamp connection formation. These events are under the strict control of two series of incompatibility alleles, the A series and the B series (Raper, 1966b). Thus, the dikaryon will result only from a mating in which the two homokaryons possess unlike A and B factors, i.e. A1 B1 X A2 B2 → (A1 B1 + A2 B2).

There is evidence from several laboratories that the incompatibility factors control specific processes necessary for the formation of the dikaryon. In matings between homokaryons having A-factors in common (common-A mating, A1 B1 X A1 B2), karyogamy and nuclear migration occur but clamp connection formation and therefore conjugate nuclear division are disrupted. In matings involving homokaryons with like-B factors (common-B mating, A1 B1 X A2 B1), clamp connection formation and conjugate nuclear

division proceeds but nuclear migration is severely restricted. Observations such as these on the effect of the incompatibility factors upon the mycelial morphology of the particular heterokaryon support the hypothesis that the A locus controls clamp connection formation and that the B locus controls nuclear migration (Esser and Raper, 1965).

Virtually nothing is known about the time in the mating process at which the incompatibility factors act. In addition, almost all the previous studies on the incompatibility system in the tetrapolar Basidiomycetes were based on observations of nuclear behavior only. The role the cytoplasm plays in the initial mating interactions of Schizophyllum commune has not been investigated. There is some evidence, however, that the cytoplasm may be involved in the initial incompatibility response. Raper (1966b) postulated a cytoplasmic effect in the unusual growth shown by heterokaryons possessing modified A and B factors. Casselton and Lewis (1967) have presented evidence that individual nuclei existing separately in the same cell behave differently from similar nuclei combined as a diploid nucleus. Also, Sicari and Ellingboe (1967) demonstrated that marked changes in the cytoplasm may occur shortly after hyphal anastomosis.

The objective of this research was to investigate the early mating interactions on the biological and molecular levels in an attempt to determine the time in the mating process at which the incompatibility factors act and the role the cytoplasm may play in initial mating interactions. More specifically, experiments were designed to: (1) determine the kinetics of initial nuclear exchange in compatible and non-compatible matings; and (2) to determine whether the cytoplasms are exchanged in matings, at which time such exchange might occur, and the possible manifestations of exchange. In connection with these studies, preliminary

experiments were conducted to determine whether intact functional ribosomes could be isolated from Schizophyllum commune and Coprinus lagopus.

LITERATURE REVIEW

Sexual incompatibility in the higher fungi has been studied intensively since the original investigations of Kniep (1913a,b). The researches on incompatibility were reviewed extensively in recent years (Lewis, 1954; Papazian, 1958; Raper, 1966a, b; Raper and Esser, 1964; Raper and Raper, 1968). Much of the interest has centered on the Basidiomycetes Schizophyllum commune and Coprinus lagopus.

The incompatibility system in Schizophyllum and Coprinus determines the events in the life cycle of these fungi. Comprehensive accounts of the life history of Schizophyllum and Coprinus have been published (Buller, 1941; Whitehouse, 1949; and Raper, 1953). The binucleate basidiospore germinates to produce the homokaryon consisting primarily of uninucleate cells (Jersild, Mishkin and Niederpruem, 1967). The homokaryon is the haploid phase of the life cycle and is capable of indefinite vegetative growth. The second phase of the life cycle is termed the dikaryotic phase or dikaryon. The dikaryon must result from the sexual mating of two homokaryons having compatible mating types.

The incompatibility system in Schizophyllum and Coprinus has been designated as tetrapolar incompatibility by Kniep (1920). Tetrapolarity refers to the fact that mating type in these fungi is determined by two factors, A and B, which segregate independently at meiosis (Kniep, 1920). The A factor of Schizophyllum commune consists of two closely-linked genes (subunits) $A\alpha$ and $A\beta$ (Papazian, 1951; Raper, Baxter and Middleton,

1958; Raper, Baxter and Ellingboe, 1960). The number of A factors was shown to be approximately 500, with 9 $A\alpha$ alleles and 50 $A\beta$ alleles estimated (Raper and Raper, 1968). The B-factor is also complex and consists of at least two loci, $B\alpha$ and $B\beta$. There appears to be fewer B factors in the world-wide population, with a total of 93 estimated. To date, 7-8 $B\alpha$'s and 7 $B\beta$'s have been identified (Koltin, Raper and Simchen, 1967; Raper and Raper, 1968). The dikaryon is normally established from a mating between homokaryons having different A and different B factors. Only one of the α and β subunits of each of the A and B factors must be different for a compatible relationship. For example, the mating $A(\alpha 1\text{-}\beta 1)\ B(\alpha 2\text{-}\beta 2) \times A(\alpha 2\text{-}\beta 1)\ B(\alpha 2\text{-}\beta 2)$ will produce a dikaryon, whereas $A(\alpha 1\text{-}\beta 1)\ B(\alpha 1\text{-}\beta 1) \times A(\alpha 1\text{-}\beta 1)\ B(\alpha 2\text{-}\beta 2)$ will not. Complex A and B factors thought to be of a similar nature have been identified in other tetrapolar Basidiomycetes (Day, 1960; Terakawa, 1960; Takemaru, 1961).

The mechanism of action of the incompatibility factors has been studied primarily through observations of the biological effects of the factors on heterokaryosis. Heterokaryosis was defined (Hansen and Smith, 1932) as the condition in which two (or more) different nuclear types can be shown to have co-existed in a single hypha, cell, or multinucleate conidium. The method most commonly used to induce heterokaryosis is to employ two strains whose nuclei carry non-allelic, complementing mutations. The resulting mycelium will appear much like the wild-type, Dodge (1942) showed that two slow-growing strains of Neurospora can form a rapidly growing heterokaryotic mycelium. Beadle and Coonradt (1944) showed that complementation between two non-allelic nutritional requirements would permit the heterokaryon to grow as a stable prototroph. Nuclear complementation of auxotrophic mutations has become a standard

method for studying heterokaryosis in the fungi, bacteria, and higher organisms such as Drosophila (Fincham, 1966).

A complex series of events has been shown to be necessary for successful establishment of the dikaryon in Schizophyllum and Coprinus. The sequence of events can be summarized as follows (Raper and Raper, 1968):

1. Hyphal anastomosis and nuclear exchange: Anastomosis occurs between two homokaryotic mycelia and reciprocal exchange of nuclei follows. Hyphal fusions will occur between hyphae of a single homokaryon (Raper and Miles, 1958).

2. Nuclear migration: Buller (1931) first demonstrated that the exchanged nuclei will migrate throughout the recipient mycelium. This phenomenon has been confirmed in Schizophyllum commune (Snider and Raper, 1958; Snider, 1963; Ellingboe, 1964), in Coprinus lagopus (Swiezynski, 1961a, b; Swiezynski and Day, 1960b) and in Gelasinospora (Dowding and Buller, 1940).

3. Nuclear pairing: The reciprocal exchange of nuclei and reciprocal nuclear migration results in a dikaryotic mycelium in which each cell contains two nuclei, one from each homokaryon. Some strains, designated unilateral maters, will donate nuclei but do not function as recipient mycelia (Papazian, 1951). Only the homokaryon which is bilateral will become dikaryotized.

4. Conjugate nuclear division with concomitant clamp connection formation: Kniep (1920) observed that both nuclei in the dikaryotic cell divided at the same time. One nucleus divided along the axis of the cell. The other nucleus was oriented so that one daughter nucleus migrated through the clamp connection. This process insured that the new tip

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cell would become dikaryotic and the subterminal cell would remain so. Raper and Raper (1968) subdivide clamp connection formation into 3 distinct events: formation of the hook-cell, formation of septations in the apical cell and in the hook-cell, and fusion of the hook-cell with the subterminal cell. The division was based on the demonstrated effects of mutations of the A and B factors on the formation of clamp connections (Raper and Raper, 1966).

In Schizophyllum commune, heterokaryons resulting from incompatible matings are morphologically distinguishable, making them a particularly useful tool.

The common-A or "flat" heterokaryon results from a mating such as A1 B1 X A1 B2 in which both homokaryons have a common-A factor. Papazian (1950) demonstrated that reciprocal nuclear exchange and nuclear migration occur in common-A matings as in compatible matings. The important features of the common-A mating are that the nuclear ratios in the individual cells are very irregular, ranging from near 1:1 to 4000:1 (Snider and Raper, 1965), that such disparate nuclear ratios have marked effects on nuclear complementation (Raper and San Antonio, 1954) and, the almost total absence of clamp connections (Papazian, 1950).

The common-B heterokaryon, derived from a mating such as A1 B1 X A2 B1, was first described by Quintanilha (1935) and was later confirmed by Papazian (1950). The distinctive feature of the common-B heterokaryon is the presence of false clamp connections (Parag, 1960). Hook cells develop as in the dikaryon but fail to fuse with the subterminal cell, so that one nucleus becomes trapped in the hook cell. The terminal cell is binucleate (dikaryotic) but the subterminal cell is uninucleate (homokaryotic). Growth of the unfused hook-cell or lateral branching of

the subterminal cell produces homokaryotic hyphae.

Heterokaryosis in the common-AB mating (A1 B1 X A2 B2) was demonstrated through nuclear complementation between homokaryons possessing different auxotrophic mutations (Middleton, 1962, 1964). Only a small portion of the established mycelium became truly heterokaryotic, with nuclear ratios at the point of inoculum near 1:1, but, at the mycelial periphery, both types of nuclei were found together very rarely.

Studies of heterokaryosis in Schizophyllum commune and other tetrapolar species have led to the proposal that the incompatibility factors control specific events of the sequence leading to the successful establishment of the dikaryon. It was suggested that the A factor controls nuclear migration and the B factor controls clamp connection formation (Esser and Raper, 1965; Raper, 1966b). Additional support for these hypotheses has been obtained from the study of A and B factor mutants and modifier mutations. These studies were discussed at length in recent reviews (Raper, 1966b; Raper and Raper, 1966). This account will therefore be quite brief. Homokaryons possessing such mutated factors mimic heterokaryons in basic morphology, nuclear distribution, cellular nuclear ratios, and mating behavior (Raper, Boyd, and Raper, 1965). Modifier mutations of five distinct phenotypes have been isolated and described (Raper and Raper, 1964; Raper and Raper, 1966). Each of these classes of modifiers may effect a particular step in dikaryosis or they may effect several steps. The ultimate effects of experiments with modifier mutations have been a greater understanding of the sequence of events necessary for the establishment of the dikaryon and greater elucidation of the role of the individual A and B factors in this sequence.

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Various models for the mode of action of the incompatibility factors have been proposed. These were summarized in the recent reviews by Lewis (1966), Raper (1966b) and Esser and Raper (1965). Only two of the more recent models will be discussed here. Lewis (1964) proposes a protein dimer basis for pollen-style incompatibility in higher plants. The product of the S and Z genes is hypothesized to be a dimer, one polypeptide chain, α, coded for by the S gene and the other polypeptide, β, coded for by the Z gene. The particular S and Z alleles give specific dimers. This hypothesis is in accord with genetic evidence (Lewis, 1960). In addition there is biochemical evidence in support of the dimer hypothesis. Lewis (1964) summarized this evidence as: a specific pollen protein, a specific style protein, a complex pollen-style protein (all identified by serological tests), and a unique RNA species found in styles pollinated with incompatible pollen. This RNA is not present before pollination. The RNA is thought to be the product of an induced gene and to code for an inhibitor of pollen tube growth.

The model for incompatibility in the Basidiomycetes proposed by Dick (1965) and Raper (1966b) also hypothesizes "incompatibility proteins" which are dimers. One polypeptide chain of the dimer is produced by the α-subunit, the other by the β-subunit. These authors postulated that the "incompatibility proteins" act in the homokaryon to repress the operon (O) responsible for initiating gene action necessary for dikaryosis. The compatible mating reaction occurs because the dimers of one mate bind to the dimers of the other forming a tetramer which cannot act as a repressor. The basis for this model is the genetic evidence derived from studies of heterokaryosis and of modifier mutations. Raper and Esser (1961) demonstrated by serological techniques

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specific differences between the proteins of two isogenic homokaryons and the dikaryon that resulted from their mating. Similar differences were demonstrated by acrylamide-gel disc electrophoresis by Dick (1965). However, differences of the same magnitude were demonstrated between homokaryons.

Recently, Wessels and Niederpruem (1967) reported that a cell-wall glucan-degrading enzyme isolated from Schizophyllum commune (Wessels, 1966) may play a role in the mating process. On the basis of data which indicated that enzyme activity was greater in dikaryons and common-A heterokaryons than in the component homokaryons, the authors postulated that unlike B factors are necessary for derepression of the enzyme. However, their data also indicated that the enzyme activity was as elevated in the common-B heterokaryons as in the common-A heterokaryon. Also, the ages of the matings were always greater than 160 hr, so that the effect of the enzyme was noted only after the particular heterokaryon was well established. A role of this enzyme in early mating interactions could not be postulated from these studies.

Very little work has been done on the molecular basis of incompatibility in the tetrapolar Basidiomycetes. Virtually nothing has been reported concerning the nature of the protein synthesis system except a characterization of the ribosomes (Taylor and Storck, 1964) and ribosomal RNA (Taylor, Glasgow, and Storck, 1967) from Schizophyllum commune. Some of the problems encountered in such studies with Schizophyllum which have deterred previous workers are the very resistant cell wall (Wang and Miles, 1966; Wessels, 1965) and the production of large amounts of extracellular polysaccharide (Dick, 1965).

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It has been shown conclusively that protein synthesis occurs on the polysomes (Goodman and Rich, 1963; Risebrough, Tissieres, and Watson, 1962; Warner, Knopf and Rich, 1963; Wettstein, Staehelin, and Noll, 1963). The isolation of polysomes has been accomplished from such diverse biological species as bacteria (Das, Goldstein and Lowney, 1967; Dresden and Hoagland, 1965; Oppenheim et al, 1968), slime molds (Phillips, Rich and Sussman, 1964), algae (Gnanam and Kahn, 1967), higher plants (Chen and Wildman, 1967) and a variety of animal tissues (Breillatt and Dickman, 1966; Infante and Nemer, 1967; Howell, Loeb, Tomkins, 1964). Polysomes have been isolated from various species of fungi (Chao and Schachman, 1956; Hanney and Storck, 1964; Kimura, Ono and Yanagita, 1965; Kuntzel and Noll, 1967; Staples and Bedigian, 1967; Staples, Bedigian, and Williams, 1968, Zalokar, 1960a,b; Zalokar, 1961a,b). However, in all the examples of polysome isolation, the source was fresh or frozen tissue and the need for very gentle means of cell disruption was stressed because of the fragility of the polyribosome-messenger RNA complex. If such methods were not applicable to Schizophyllum commune and Coprinus lagopus because of the difficulty in rupturing the cells, other methods would have to be developed.

The models for the mechanism of action of the incompatibility factors in Schizophyllum would of necessity involve the cytoplasm of the mating partners. Any "incompatibility protein" synthesized in the homokaryon would be expected to reside in the cytoplasm, either free or associated with some organelle. This suggests that investigation of the molecular basis of incompatibility should include a study of possible cytoplasmic exchange during mating and the biochemical events occurring in the cytoplasm early in the mating process. Very little is known

to date about the role of the cytoplasm in incompatibility in Schizophyllum commune or other tetrapolar Basidiomycetes. Such is not the case in the Ascomycetes. In Neurospora, in addition to the nuclear mating-type loci, there are additional genes which determine the capability of forming the heterokaryon (Sansome, 1946). Homokaryons with the same mating-type (sexually incompatible) will form the vegetative heterokaryon, whereas homokaryons of unlike mating-type (sexually compatible) will not. The genetic control of heterokaryosis resides in the CDE genes in Neurospora (Garnjobst, 1953; Garnjobst, 1955; Wilson and Garnjobst, 1966). Any two strains carrying different genes at any of the 3 loci will not form the heterokaryon. For example, a mating of CDE X CDE or cde X cde will result in a heterokaryon but cde X CDE or cDE X CDE will not. The site of action of these genes was shown to be the cytoplasm. Hyphal anastomosis is not prevented but shortly after fusion, death of one or both cells involved occurs, usually in the first hour (Garnjobst and Wilson, 1956). In microinjection studies, Wilson has demonstrated that the injection of CDE incompatible cytoplasm into cells of another strain had the same effect as hyphal fusion (Wilson, 1961; Wilson, Garnjobst, and Tatum, 1961). A substance has been isolated from Neurospora cytoplasm with some of the properties of a protein and which, when injected into cells of unlike CDE genotype, will cause death of the cells (Wilson, et al, 1961). The authors suggest that the substance may act antigenically. Heterokaryon incompatibility of a similar nature has also been demonstrated in Aspergillus by Grindle (1963).

The heterokaryon incompatibility system in Neurospora suggests that the incompatibility mechanism is constitutive, that the molecular basis of this type of incompatibility resides in the cytoplasm, and that

cytoplasmic exchange between mating partners is necessary for the incompatibility reaction to occur. The role of the cytoplasm in incompatibility has been suggested from other research. Harder (1927) used microsurgery to destroy the terminal and clamp cells of dikaryons of Schizophyllum commune. The uninucleate cells formed false clamps and eventually reverted to homokaryotic morphology. He interpreted these results to mean that some cytoplasmic substance responsible for clamp-connection formation was formed by the two nuclei of the dikaryon. This substance was thought to persist until diluted out by subsequent growth. Apirion (1966) working with Aspergillus nidulans and Casselton and Lewis (1967) with Coprinus lagopus have shown that genetic complementation of auxotrophic mutations occurs less readily in heterokaryons than in the dikaryon or diploid. Papazian (1956) suggested that the behavior of unilateral maters in Schizophyllum and other species is determined by the state of the cytoplasm. He postulated that the donors (unilateral maters) have a deficient cytoplasm not capable of supporting the foreign nuclear type. Most recently, Sicari and Ellingboe (1967) presented observations which would suggest that the presence of two nuclei in the heterokaryotic cytoplasm induces a response which results in lysis of the cells involved in anastomosis. The notable difference between these results and the situation in Neurospora was that several hours were required before any visible response was noted.

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MATERIALS AND METHODS

Strains and Media

All cultures used throughout this research had the common parent, wild-type strain 699 (Ellingboe and Raper, 1962a,b) and differed only in mating-type factors and biochemical mutations. The media used throughout were migration complete medium (Snider and Raper, 1958) and Schizophyllum minimal medium (Raper and Miles, 1958).

Mating Procedures

The homokaryons were grown on migration-complete agar medium for 5 days at 22°C. The entire 5-day-old mycelium was macerated in approximately 25 ml sterile distilled water and 5 ml portions pipetted into 300 ml Erlenmeyer flasks containing 25 ml migration complete medium. After 48 hr growth without agitation at 32°C, the homokaryotic cultures which gave the desired mating type combination were combined in a Waring Blendor cup and macerated together for 1 min in 30 sec intervals. Five ml aliquots of this mating mixture were pipetted into 25 ml of the appropriate liquid medium. The matings were incubated at 32°C without agitation for the 12-96 hr period. At the time of analysis, the mycelium was macerated for 1 min in 30 sec intervals and these fragments analyzed for the presence of the nuclei from both homokaryons.

Analysis of Nuclear Exchange

Analysis of the percentage of fragments possessing both types of nuclei was made by one or more of 3 methods: (1) The macerated

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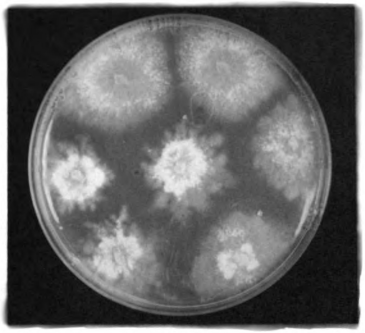
fragments from matings involving fully compatible homokaryons were examined directly by phase contrast microscopy for an estimate of the percentage of fragments having clamp connections. (2) Fragments derived from a mating and allowed to grow several days exhibit the characteristic morphology if dikaryotic or common-A. The percentage of fragments which gave rise to dikaryotic or common-A morphology after a certain period of mating was used as an estimate of the extent to which hyphal anastomosis and nuclear exchange had occurred. To make such an analysis, macerated fragments were diluted to give approximately 20-30 fragments/ml and 1 ml aliquots were added to 2 ml molten migration-complete agar medium. The entire 3 ml mixture was overlayed on plates of migration-complete agar medium. After 24 hr at 22°C, growing fragments were isolated and inoculated onto fresh agar. Fragments were allowed to grow 7 days and the morphology scored at that time (Figure 1). (3) Nuclear exchange in all four classes of matings was measured by the percentage of fragments capable of growth on minimal medium due to nuclear complementation of different auxotrophic mutations present in the two homokaryons. Nuclear complementation would be possible only if the fragments contained both types of nuclei. Fragments from the developing heterokaryon were diluted to give 20-30 fragments/ml and 1 ml aliquots were pipetted into 2 ml molten minimal medium. The entire 3 ml mixture was overlayed on plates of minimal medium. The plates were incubated at 22°C for 72-96 hr at which time they were scored for the percentage of total fragments capable of growth on minimal medium. The auxotrophic mutations used were non-leaky so that analysis of growth vs. no growth was very clear (Figure 2).



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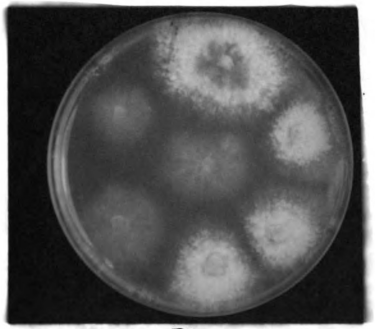

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Figure 1. Morphology of colonies derived from isolated fragments incubated 7 days at 22°C. In (A) four dikaryotic fragments are easily differentiated from three homokaryotic colonies and in (B) 3 "flat" common-A colonies can be distinguished from 4 homokaryotic colonies.

Figure

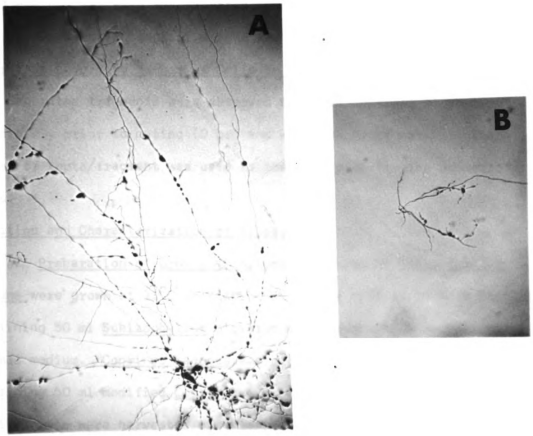

Figure 2. Growth of fragments derived from a fully-compatible mating between two different auxotrophic strains. The growing fragment (A) is clearly distinguished from the non-growing fragment (B) after 72 hr growth on minimal medium.

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b. Preparation of Spores and Germlings: Fruiting bodies of Schizophyllum commune deposited spores onto dry Petri dish covers. Spores were collected from the covers by flooding with sterile distilled water and by scraping the spores off the cover. Ungerminated spores (0 hr) were immediately centrifuged at 12,000 X g (Sorvall SS-1 centrifuge) for 5 min at 4°C, the pellet resuspended in 2 ml 5.0 mM Tris-HCl (pH 7.4) containing 5.0 mM $MgCl_2$ (Tris-Mg^{+2} buffer) and centrifuged again. The washed pellet was immediately lyophilized and subsequently ground as described above. To obtain germinating spores, the ungerminated spores were collected as described above and inoculated into flasks containing 25 ml Schizophyllum minimal medium supplemented with 0.1 mM arginine, nicotinic acid and adenine (supplemented minimal medium). The spores were germinated in shake culture at 22°C for periods up to 15 hr. The germlings and remaining ungerminated spores were collected and treated in the same manner as described for 0 hr spores. Conditions for obtaining ^{14}C-arginine labelled germlings were the same as those above except that each flask of liquid medium contained 1 μc sterile ^{14}C-arginine.

c. Preparation of Ribosomes: Predetermined amounts of ground, lyophilized material were suspended in 5 ml Tris-Mg^{+2} buffer with sodium deoxycholate added to a concentration of 1% and centrifuged at 15,000 X g for 25 min. The supernatant was layered over 6 ml 2 M sucrose (Breillatt and Dickman, 1966), the final volume brought to 12 ml with Tris-Mg^{+2} buffer and centrifuged at 105,000 X g for 3 hr. The supernatant fluid was discarded and the pellets resuspended in 2% sucrose in Tris-Mg^{+2} buffer. The non-ribosomal material was removed by centrifuging the suspension at 15,000 X g for 15 min. The resulting

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Twice-washed (2X) ribosomes were obtained by layering the 1X ribosomes over 2M sucrose and centrifuging for 3 hrs at 105,000 X g. The pellets were stored without resuspension at -17°C overnight. All operations were performed in the cold (0-4°C).

d. Characterization of Ribosomes: A 1 ml sample containing between 0.5 - 3.0 mg ribosomes was layered on either 5-20% or 15-30% linear sucrose density-gradients and centrifuged for 3.5 hr at 25,000 rpm (63,581 X g) in an SW 25.1 rotor. Distribution of ribosomes in the gradients was determined by puncturing the bottom of the tube and pumping the material through a Gilford spectrophotometer with continuous recording of the absorbancy profile at 260 mμ. If radioactivity was to be measured, consecutive 1 ml fractions were collected into scintillation vials after passing through the spectrophotometer, 15 ml Bray's scintillation fluid (Bray, 1960) added, and the radioactivity measured in a Packard liquid scintillation spectrometer. The fungal ribosome density-gradient distribution was compared to rabbit reticulocyte ribosomes (1X) centrifuged as a separate sample in each experiment.

e. Amino Acid Incorporation: The following incubation mixture, in millimoles, was prepared for the study of amino acid incorporation in vitro (Allen and Schweet, 1962): 0.25 GTP, 1.0 ATP, 5.0 PEP, 0.004 PEP-kinase, 20.0 GSH, 4.0 $MgCl_2$, 50 Tris HCl buffer (pH 7.5), 0.05 mixture of 19 amino acids excluding valine, 6 mg 40-70% rabbit blood enzyme fraction which also includes tRNA, and 0.05 of uniformly labelled L- ^{14}C-valine (1.0 μc). The final K^+ concentration was adjusted to .05 M with 1.0 M KCl. The 2X ribosomes were added and

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the final volume adjusted to 1.0 ml with deionized water. Following incubation at 37°C for 40 min, the reaction was stopped by adding 5 ml 5% (w/v) trichloroacetic acid (22°C) and 15 mg carrier bovine serum albumin. Further treatment was according to the method of Casjens and Morris (1965). The radioactivity was assayed in thixotropic counting solution (Casjens and Morris, 1965) in a Packard liquid scintillation spectrometer.)

f. Equilibrium Density-Gradient Centrifugation: Ribosomes isolated from Schizophyllum and Coprinus vegetative mycelia were subjected to cesium chloride density-gradient centrifugation. Once-washed ribosomes resuspended in Medium B (Allen and Schweet, 1962) were brought to a volume of 3.0 ml and 4.0 g CsCl added with rapid stirring to give a starting density of 1.73 g/cc. Four ml of this mixture were placed in the centrifuge tube and mixed again with a vibrator stirring apparatus. The samples were centrifuged for 18 hr at 40,000 rpm (130,576 X g), 10°C, in the SW50L rotor of the Spinco ultracentrifuge. The tubes were punctured under conditions insuring controlled flow rate and 8-10 drop fractions collected automatically. The refractive index of the undiluted samples was determined with a Bausch and Lomb refractometer and the density calculated from the equation: $\rho^{20} = 10.860 \times \text{refractive index} - 13.4921$ g/cc (Ifft, Voet, and Vinograd, 1961). The density at a particular band was estimated by interpolation. The fractions were diluted to a minimum volume of 0.3 ml and the absorbancy at 260 mμ assayed in a Beckman DU spectrophotometer.

Localization of Radioactive Label

The disappearance of ^{14}C-arginine from the culture medium and the distribution of the radioactive amino acid which was taken up into the

various fractions of the fungus was determined for germinating spores, homokaryotic mycelium, and mycelium derived from matings between a homokaryon grown in D_2O-medium and a homokaryon grown in H_2O-medium containing ^{14}C-arginine.

a. Uptake of Radioactivity from the Culture Medium: Germlings labelled with ^{14}C-arginine were prepared as described earlier. At 0-8 hr after inoculation, three 2 ml samples were removed from the same flask and centrifuged for 5 min at 10,000 X g. One ml samples of the supernatant were placed in scintillation vials, 15 ml Bray's scintillation fluid added, and the samples counted in a Packard liquid scintillation spectrometer. At 1, 6, 9, 12, and 15 hrs after inoculation, the germinating spores were harvested for further analyses and 1 ml samples of the culture medium supernatant of the 9, 12, and 15 hr germlings were treated in the manner just described.

Homokaryotic mycelium was grown in supplemented minimal medium in still culture for 48 hr at 32°C. The mycelium was macerated for 1 min in a Waring Blendor cup and 25 ml aliquots inoculated into supplemented minimal medium containing 1 μc sterile ^{14}C-arginine. The cultures were incubated for 24 hr in shake culture at 22°C. At 3, 6, 9, 18, 20, and 24 hr after inoculation, 2 ml samples were removed and treated in the same manner as described for germinating spores. At 24 hr, half the cultures were harvested, lyophilized, and ground. The remaining cultures were centrifuged under sterile conditions at 10,000 X g for 15 min, the pellet resuspended in 10 ml sterile water, and centrifuged again. This wash was repeated twice. The washed pellet was resuspended in 10 ml sterile water and the entire contents inoculated into a flask containing 25 ml supplemented minimal medium. These

cultures were incubated for 24 hr in shake culture at 22°C, harvested, lyophilized, and ground.

Homokaryons were grown in medium containing ^{14}C-arginine prior to mating with deuterium (D_2O) labelled homokaryons. The amount of radioactivity remaining in the medium was assayed after each period of growth in radioactive medium. The amount of radioactivity in the wash supernatants and in the culture medium in which the mating was made was also determined after the mating period.

b. Radioactivity in 15,000 X g Pellet: Predetermined amounts of ground mycelium were resuspended in Tris-Mg^{+2} buffer for preparation of ribosomes. The 15,000 X g pellet was resuspended in buffer, aliquots placed in scintillation vials and dried at 100°C for 1 hr. Fifteen ml Toluene-POPOP scintillation fluid was added and the samples counted.

c. Radioactivity in Ribosomes: The ribosomes were prepared in the usual manner. The radioactivity in the ribosomal pellet was determined by one or more of 3 methods. The ribosomes from germinating spores were analyzed solely by sucrose density-gradient centrifugation as previously described. The ribosomes from vegetative mycelia and matings were analyzed by counting small aliquots of ribosomes in Bray's scintillation fluid and by analysis of fractions obtained from CsCl density-gradients.

d. Label in Microsomal Residue: Centrifugation of the resuspended microsomal pellet at 15,000 X g for 10 min resulted in a pellet which was resuspended in 1 ml Tris-Mg^{+2} buffer, placed in a scintillation vial and dried at 100°C for 1 hr. The radioactivity was determined in Toluene-POPOP scintillation fluid.

e. Radioactivity of the 105,000 X g Supernatant: One ml samples of the 105,000 X g supernatant from all 3 kinds of mycelia were removed and frozen until use. The radioactivity in the untreated samples was determined by counting small aliquots in Bray's scintillation fluid. The amount of radioactivity in the TCA soluble and precipitable fractions was determined by the method described for analysis of *in vitro* amino acid incorporation.

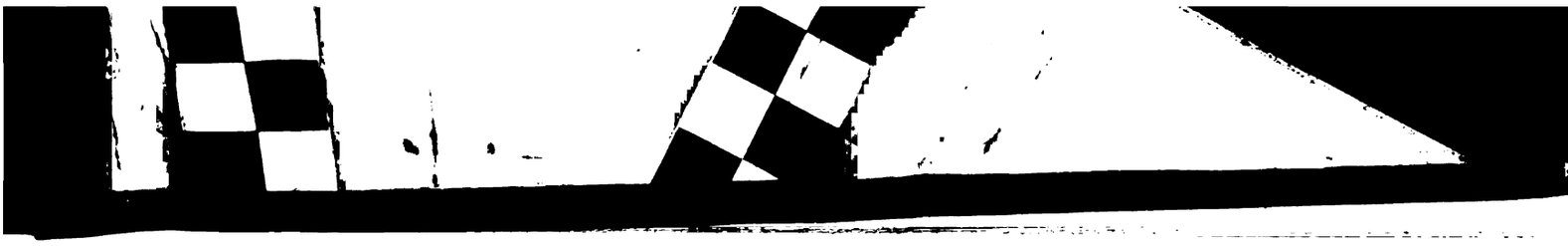
Growth of Homokaryon in Deuterium-Containing Medium

Cultures of wild-type strain 699 grown for 48 hr in liquid minimal medium were macerated for 1 min in a Waring Blendor cup. Five ml aliquots of the macerate were inoculated into flasks containing 25 ml minimal medium plus various proportions of D_2O. D_2O was substituted for 0, 25, 50, 75, and 100% of the H_2O before autoclaving. The flasks were incubated in shake culture at 22°C for five days, harvested by centrifugation, and washed three times in distilled water to remove the extracellular polysaccharide. The washed pellet was dried at 75°C until constant weight was attained.

The amount of extracellular polysaccharide produced by the cultures was used as another criterion of growth. The culture medium supernatant was decanted after centrifugation of the cultures, 3X its volume of 95% ethyl alcohol was added and the flasks allowed to stand at 22°C overnight. The polysaccharide which had precipitated was removed with a glass rod into an aluminum weighing dish and dried at 75°C until constant weight was attained.

Analysis of Cytoplasmic Exchange

a. Preparation of Mycelia: Wild-type strain 699 was grown in liquid minimal medium for 48 hr at 32°C, macerated, centrifuged at



10,000 X g for 20 min and washed twice with sterile water. The mycelial pellet was resuspended in 10 ml sterile water, inoculated into 100% D_2O minimal medium and incubated in shake culture for 48 hr at 22°C. These cultures were again macerated, centrifuged, washed as before, and the entire mycelium reinoculated into fresh 100% D_2O minimal medium. The cultures were incubated in shake culture for 72 hr at 22°C.

An arginine-requiring homokaryon, A1 B1 arg-6, was grown in liquid supplemented minimal medium for 48 hr at 32°C, macerated, centrifuged at 10,000 X g for 20 min, and washed twice with sterile water. The mycelial pellet was resuspended in 10 ml sterile water and inoculated into flasks of supplemented minimal medium containing 2-4 μc sterile ^{14}C-arginine and incubated in shake culture for 24 hr at 22°C. These cultures were again macerated, centrifuged, and washed. The entire mycelium was reinoculated into fresh supplemented minimal medium with 2-4 μc ^{14}C-arginine per flask. The cultures were incubated in shake culture for 24 hr at 22°C. The uptake of radioactivity was assayed as described earlier.

b. Mating Procedure: The homokaryotic mycelia were macerated separately for 1 min, centrifuged at 10,000 X g for 20 min, resuspended in sterile water, and centrifuged again. The wash was repeated twice. The amount of radioactivity in the culture medium supernatant and each wash supernatant of the homokaryon grown in ^{14}C-arginine was assayed. The mycelia were resuspended in sterile water, poured into a Waring Blendor cup containing 100 ml sterile water and mixed by running the blender for 3 sec. Fifteen ml aliquots of this mating mixture were inoculated into Roux bottles containing 50 ml supplemented minimal medium. The mixtures were incubated in still culture at 32°C. At 12

and 24 hr after mating, the mycelia were harvested by filtration, washed with more than 2 l of H_2O, lyophilized, and ground. The extent of mating was estimated by removing 5 ml of the mycelium prior to filtration, macerating for 1 min, and observing microscopically the percentage of fragments having clamp connections.

c. CsCl Density-Gradient Centrifugation: Ribosomes isolated from the ground mycelium were subjected to CsCl density-gradient centrifugation for 18 hr at 10°C. Eight-ten drop fractions were collected and the refractive index determined. The number of drops per fraction was constant in a single experiment and was varied only in different experiments. Subsequently, the fractions were diluted with 0.3 ml Medium B and the absorbancy at 260 mμ was assayed. Each fraction was spotted on 1 inch by 2-1/4 inch filter paper strips in scintillation vials, air-dried for at least 24 hr, 15 ml Toluene-POPOP scintillation fluid added and the radioactivity determined in a Packard liquid scintillation spectrometer. The concentration of the primary and secondary fluors was increased in this scintillation fluid to overcome quenching due to the CsCl.

RESULTS

Nuclear Exchange Analyzed by All Three Methods

The kinetics of nuclear exchange in matings between fully-compatible homokaryons having different nutritional requirements was analyzed by all three methods. The averaged results from 4 replicates of one such mating, A41 B41 arg-6 X A47 B47 nic-3 are illustrated in Figure 3. The percentage of fragments able to grow on minimal medium due to nuclear complementation and the percentage of isolated fragments having the dikaryotic morphology follow almost identical kinetics throughout the 96 hr test period. This would indicate that either method of analysis provides an accurate measure of the percentage of heterokaryotic fragments at any time after mating. The kinetics of nuclear exchange measured by direct observation of the percentage of fragments having clamp connections show a lag of about 12 hr, when compared to those kinetics measured by the other two methods. A lag is expected, since time is required for the clamp connection to form after the dikaryotic condition has been established. However, the kinetics based on all three methods show similar curves.

Of particular interest are the data obtained in the first twelve hours of mating which indicate that a significant percentage of the fragments in the mating mixture have interacted to the level of hyphal anastomosis and nuclear exchange.

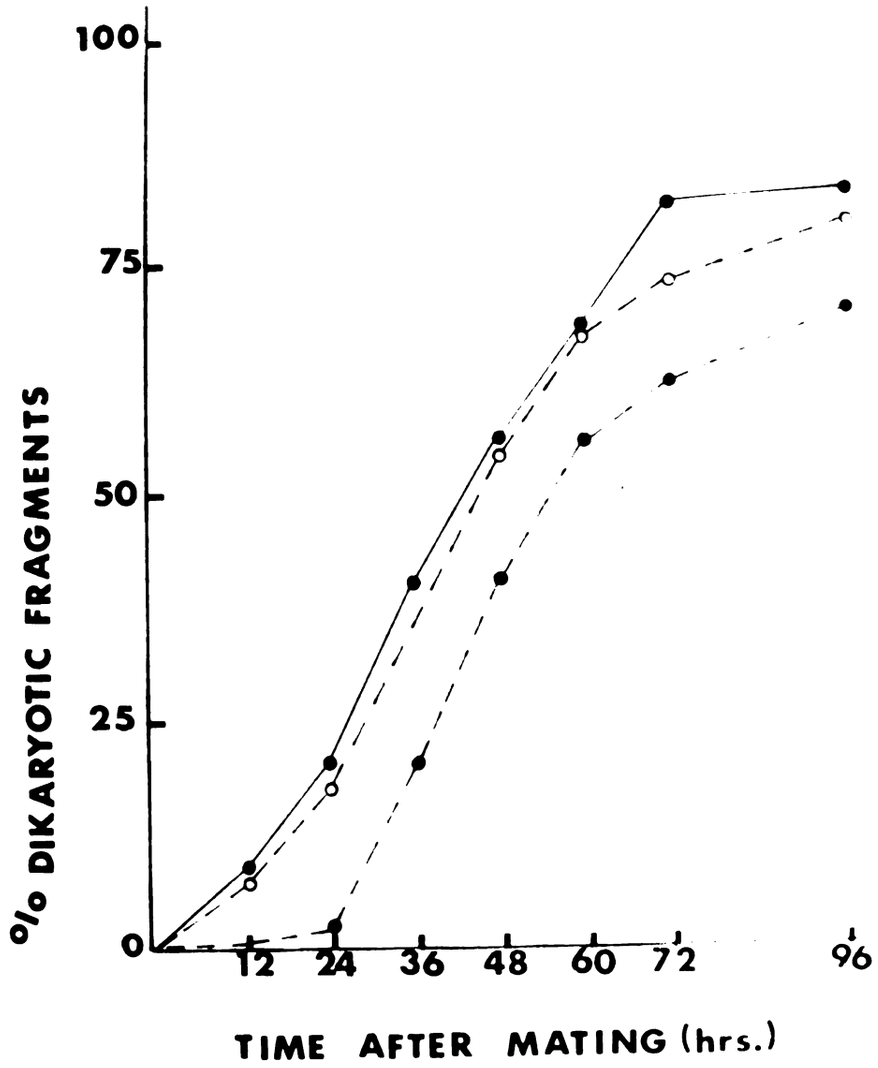


Figure 3. Kinetics of nuclear exchange in a fully-compatible mating analyzed by direct observation of clamped fragments, ●----●; dikaryotic morphology of isolated fragments, O----O; and the ability to grow on minimal medium due to nuclear complementation, ●——●.

Nuclear Exchange in All 4 Classes of Matings

The demonstration that nuclear complementation in fragments derived from a mating was a valid criterion for the analysis of the extent to which hyphal anastomosis and nuclear exchange had occurred made it possible to study the kinetics of the early mating interactions in all 4 classes of matings. This method was the only one available for the study of common-B and common-AB matings, since these two heterokaryons do not have a particular morphology which can be differentiated from the homokaryon and the heterokaryons are unstable.

The kinetics of nuclear exchange based on the percentage of mycelial fragments able to grow on minimal medium due to nuclear complementation for all 4 types of matings are shown in Figure 4. The matings illustrated are fully compatible (A41 B41 arg-6 X A42 B42 nic-2), common-A (A42 B42 arg-6 X A42 B41 nic-2), common-B (A41 B42 arg-6 X A42 B42 nic-2), and common-AB (A41 B41 arg-6 X A41 B41 nic-2). In all 4 classes of matings, the percentage of prototrophic fragments is similar in the first 12-24 hr after mating. At 24 hr, differences between the matings first become apparent. The kinetics of nuclear complementation in the fully-compatible mating appear significantly different from the other 3 classes of matings from 36 hr onward. Also, the common-A mating is distinguishable from the other classes after the first 24 hr of mating. The kinetics of nuclear complementation in the common-B and common-AB matings are quite similar. In both classes, the percentage of prototrophic fragments remains quite low throughout the 96 hr period.

These data demonstrate that there are a significant number of fragments involved in mating interactions as early as 12 hr after

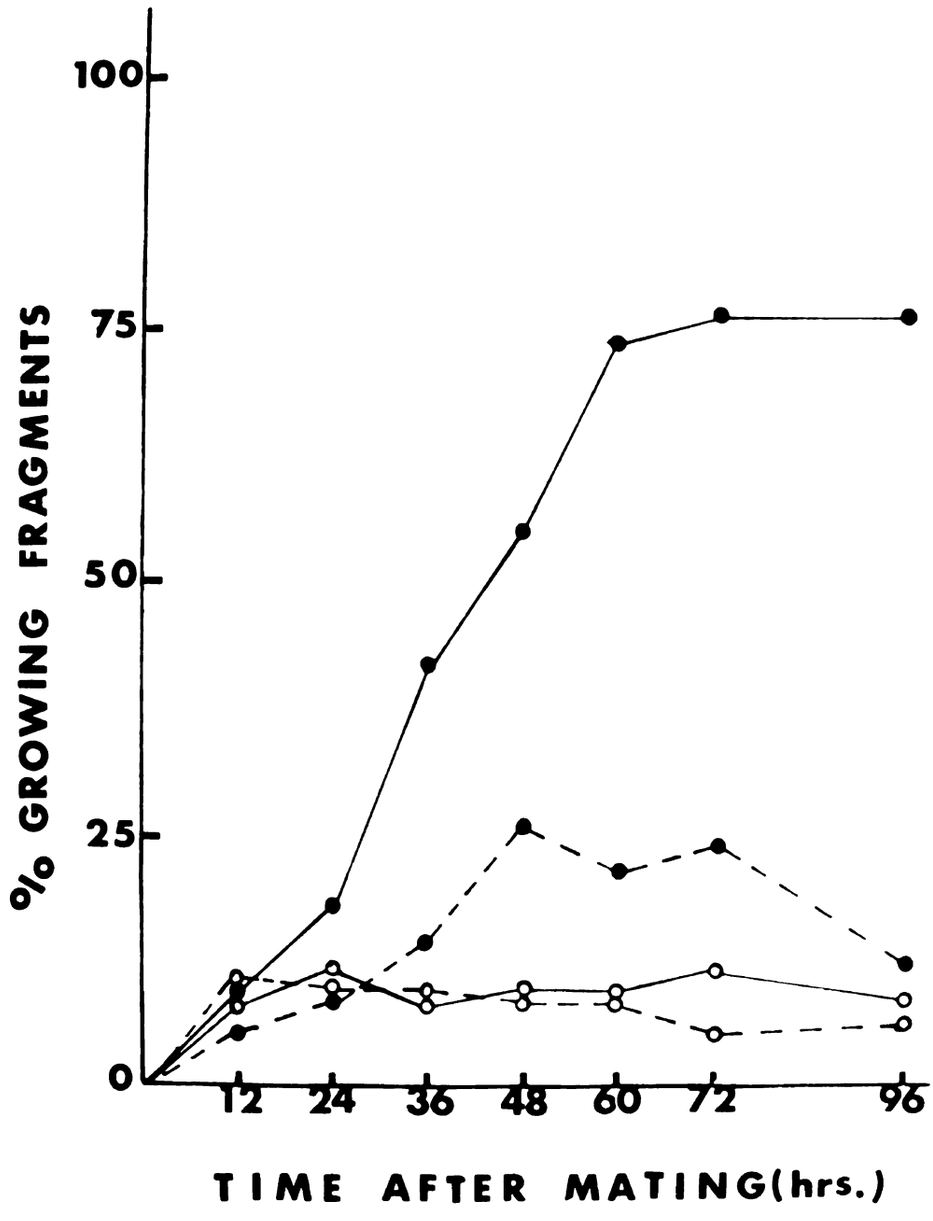


Figure 4. Kinetics of nuclear complementation in all 4 classes of matings, (O----O, common-AB mating A41 B41 arg-6 X A41 B41 nic-2; O——O, common-B mating A41 B42 arg-6 X A42 B42 nic-2; ●----●, common-A mating A42 B42 arg-6 X A42 B41 nic-2; ●—●, fully-compatible mating A41 B41 arg-6 X A42 B42 nic-2).

mating. In addition, the kinetics shown in Figures 4 and 5 would indicate that the compatibility of the mating appears to have no effect on these earliest interactions, since all 4 classes of mating have similar kinetics in the first 12-24 hrs.

Comparison of the data for the fully-compatible matings in Figures 3 and 4 shows that the kinetics of nuclear complementation are quite similar. This would indicate that neither the particular mating type alleles (A41 B41 X A47 B47 vs. A41 B41 X A42 B42) nor the particular auxotrophic mutations (arg-6 X nic-3 vs. arg-6 X nic-2) have an effect on the kinetics of nuclear exchange. This is further supported by the data presented in Figure 5. The matings represented are fully-compatible (A1 B1 arg-6 X A2 B2 nic-3). common-A (A1 B1 arg-6 X A1 B2 nic-3), common-B (A1 B1 arg-6 X A2 B1 nic-3), and common-AB (A1 B1 arg-6 X A1 B1 nic-3). It can be seen that the kinetics are essentially identical to those in Figure 4.

The differences in the percentage of prototrophic fragments after 12 hr of mating among the various classes of matings are within experimental error. These differences are not considered significant since a comparison of Figure 4 and Figure 5 reveals that the difference between the common-A and common-AB matings shown in Figure 4 is reversed in Figure 5. With a greater number of replicates, statistical analysis of these data should show the differences between the fully-compatible mating and the non-compatible matings at 24 hr and thereafter to be statistically significant. This is thought to be true because such differences were consistently obtained and the degree of difference is very reproducible.

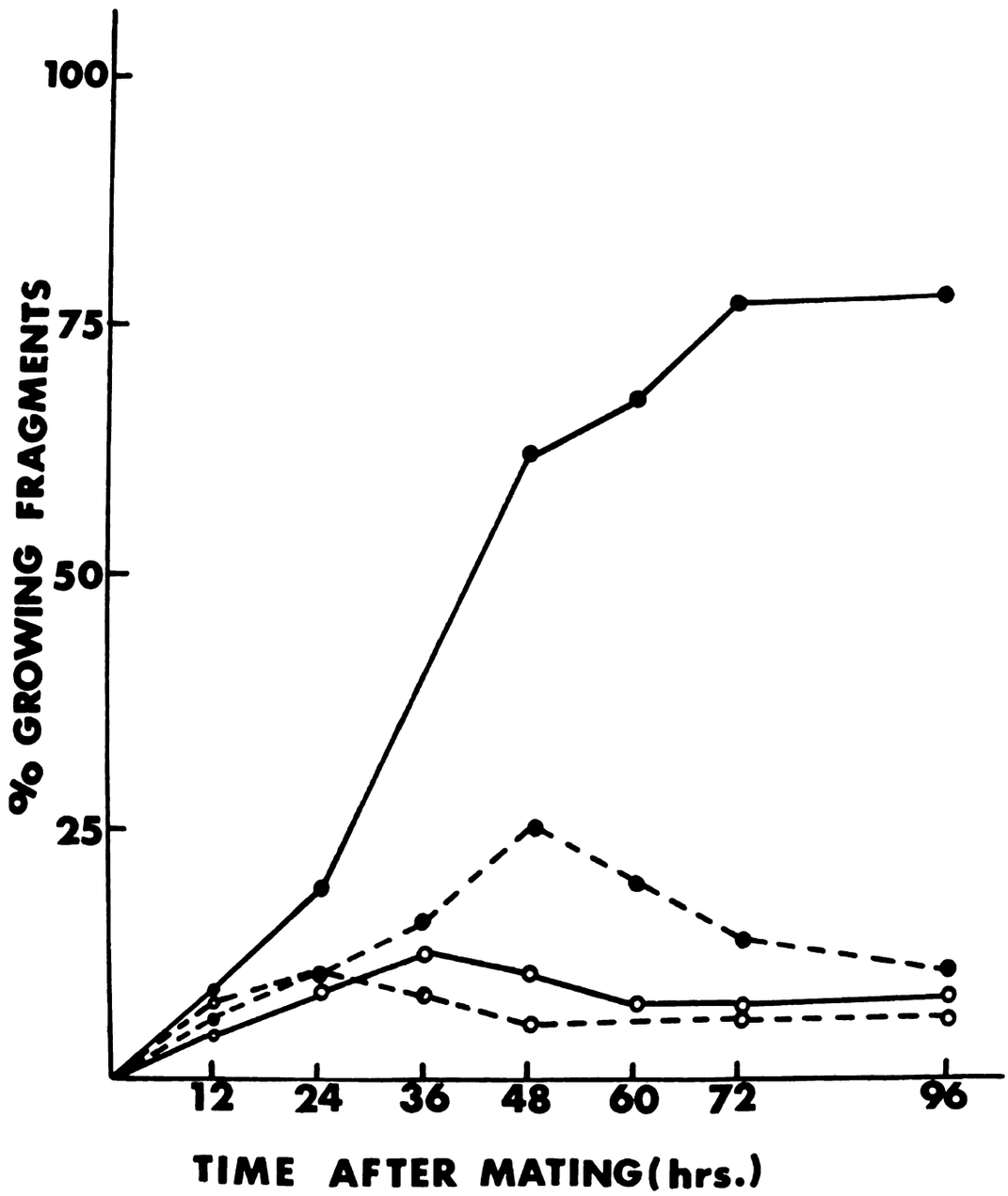


Figure 5. Kinetics of nuclear complementation in all 4 classes of matings, (O----O, common-AB mating A1 B1 arg-6 X A1 B1 nic-3; O——O, common-B mating A1 B1 arg-6 X A2 B1 nic-3; ●----●, common-A mating A1 B1 arg-6 X A1 B2 nic-3; ●——●, fully-compatible A1 B1 arg-6 X A2 B2 nic-3).

When fragments derived from a common-A mating were plated on minimal medium and analyzed for the percentage of growing fragments, an unusual type of fragment was frequently noted. These fragments, an example of which is shown in Figure 6, appeared to have begun to grow, producing a single long hypha with very few branches. To determine whether these fragments represented heterokaryotic fragments which were incapable of continued growth on minimal medium, isolated fragments were transferred to migration complete medium and the colonies which developed were scored for "flat" or common-A morphology. The results are given in Figure 7. As these data indicate, a larger percentage of fragments develop the flat morphology than are capable of growth on minimal medium. To test whether the common-A fragments were truly heterokaryotic, small portions of mycelia were transferred to minimal medium and scored for growth after 3, 4 and 7 days. In all cases, those fragments designated as common-A grew on minimal medium while none of the fragments scored as homokaryotic showed any sign of growth.

Throughout these studies, the viability of the fragments derived from the matings at the various times (0-96 hr) was checked. The percentage of the total fragments capable of growth on complete medium was used as a criterion. In all classes of matings, at least 90% of the fragments grew on complete medium after 24 hr incubation at 22°C and the percentage of viable fragments did not vary throughout the 96 hr period.

Efficiency of Mating in Different Media

Once the basic kinetics of early mating interactions were known, experiments were performed to compare the kinetics of nuclear exchange under different environmental conditions. Of particular interest was

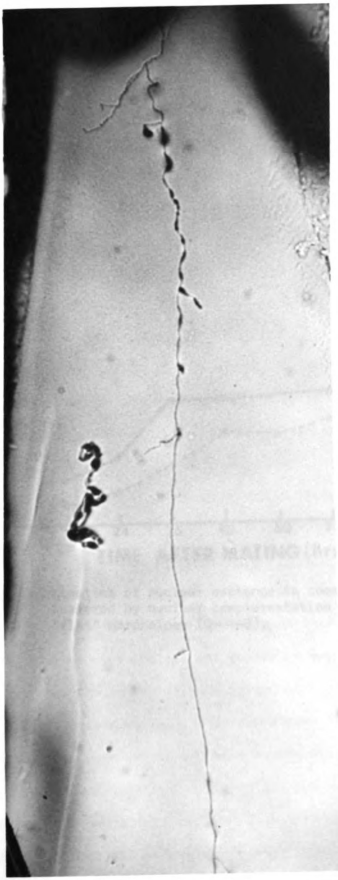


Figure 6. Example of unusual fragment in macerates of common-A mating plated on minimal medium.

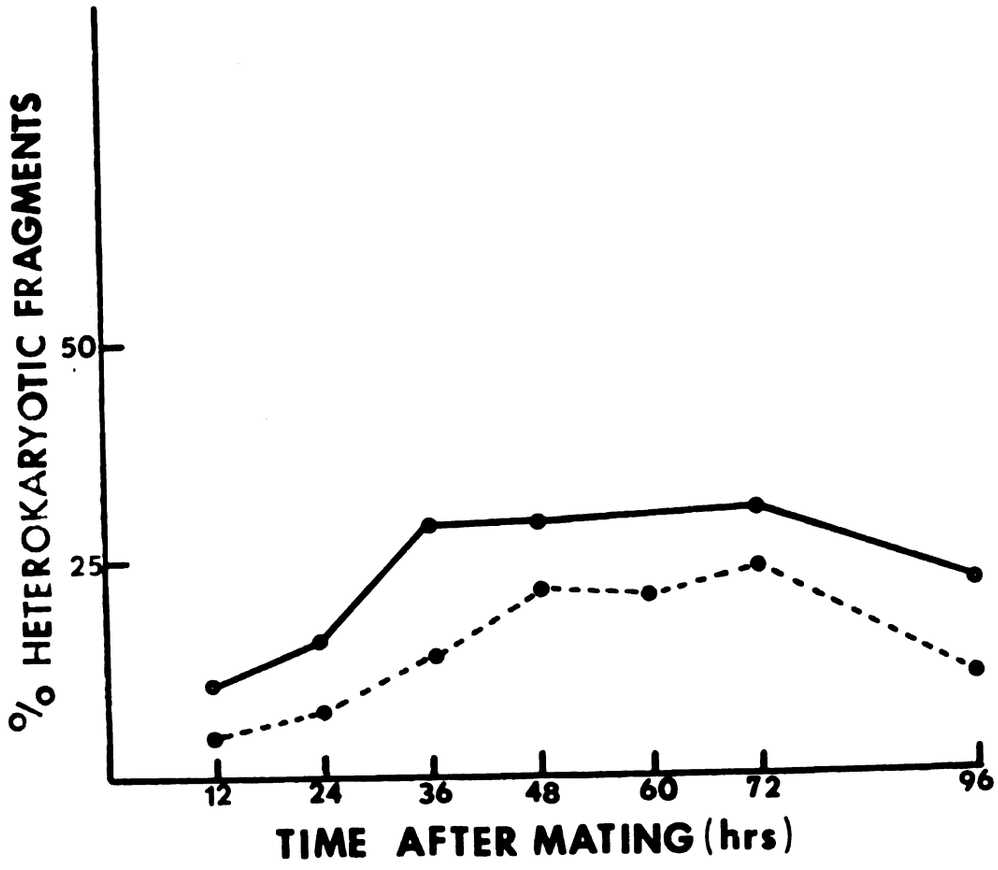


Figure 7. Kinetics of nuclear exchange in common-A matings as analyzed by nuclear complementation (●----●) and "flat" morphology (0——0).

the efficiency of early mating interactions in complete medium *vs.* minimal medium, since the latter is a defined medium useful in biochemical and molecular studies. In Figure 8, the kinetics of mating for wild-type strains *A1 B1* X *A2 B2* in complete medium and minimal medium are compared. Analysis was made by direct observation of clamped fragments and the dikaryotic morphology of isolated fragments. The kinetics of dikaryosis follow similar curves in both media, but in minimal medium the establishment of the dikaryon proceeds more rapidly after the first 24 hr of mating. The data obtained for matings made in minimal medium would indicate that the interactions are more synchronous than those in complete medium. The differences in mating kinetics do not appear to be due to differences in growth of the fragments. As indicated in Figure 9, the dry weights of mycelium produced are very similar throughout the first 60 hr, well after differences in mating kinetics are obvious. The differences in dry weight of mycelium at 72 and 96 hr are thought to be due more to inadequate removal of extracellular polysaccharide than to actual differences in mycelial mass.

Determination of the Number of Cells in the Fragments

Future research into the molecular basis of the incompatibility reaction will require information on the number of cells in fragments involved in matings when the methods described here are used. To obtain an estimate, the number of cells/fragment was counted at 0 hr and at 48 hr after a mating between fully-compatible homokaryons. At both times, the mycelia were fragmented in a Waring Blendor for 1 min. The results obtained are presented in Figure 10. It can be seen that most fragments have between 1 to 4 cells at both 0 hr and 48 hr. The percentage of fragments with more than 10 cells is very low as is the

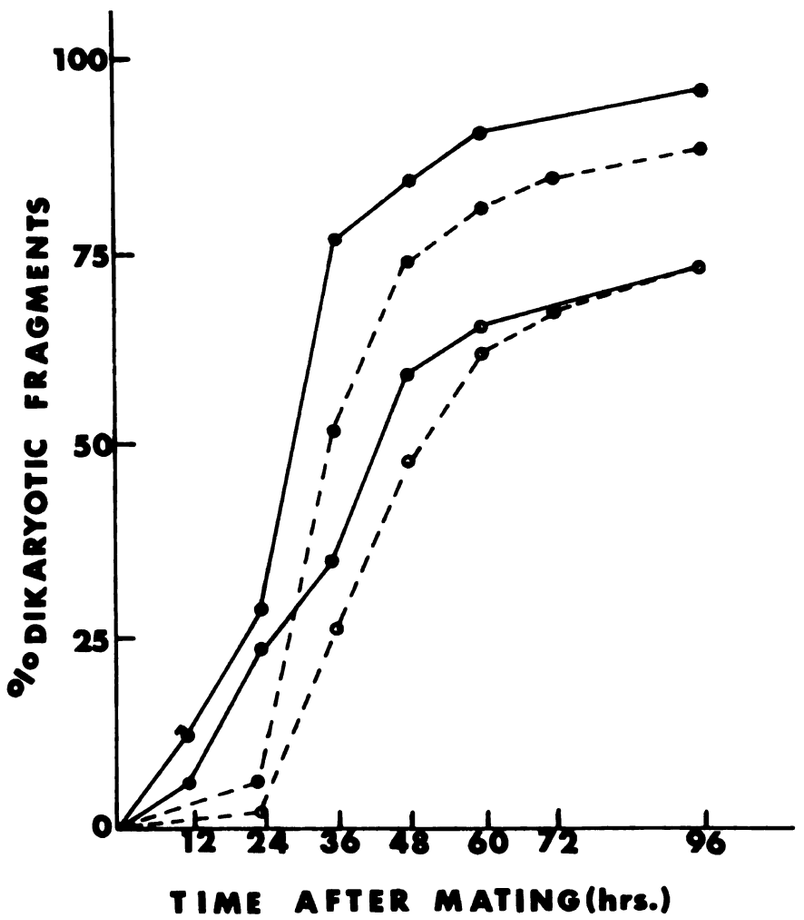


Figure 8. Comparison of kinetics of nuclear exchange in a fully-compatible mating in complete medium and minimal medium O----O, direct observation of clamped fragments from complete medium; O——O, dikaryotic morphology of isolated fragments from complete medium; ●----●, direct observation of clamped fragments from minimal medium; ●——●, dikaryotic morphology of isolated fragments from minimal medium.

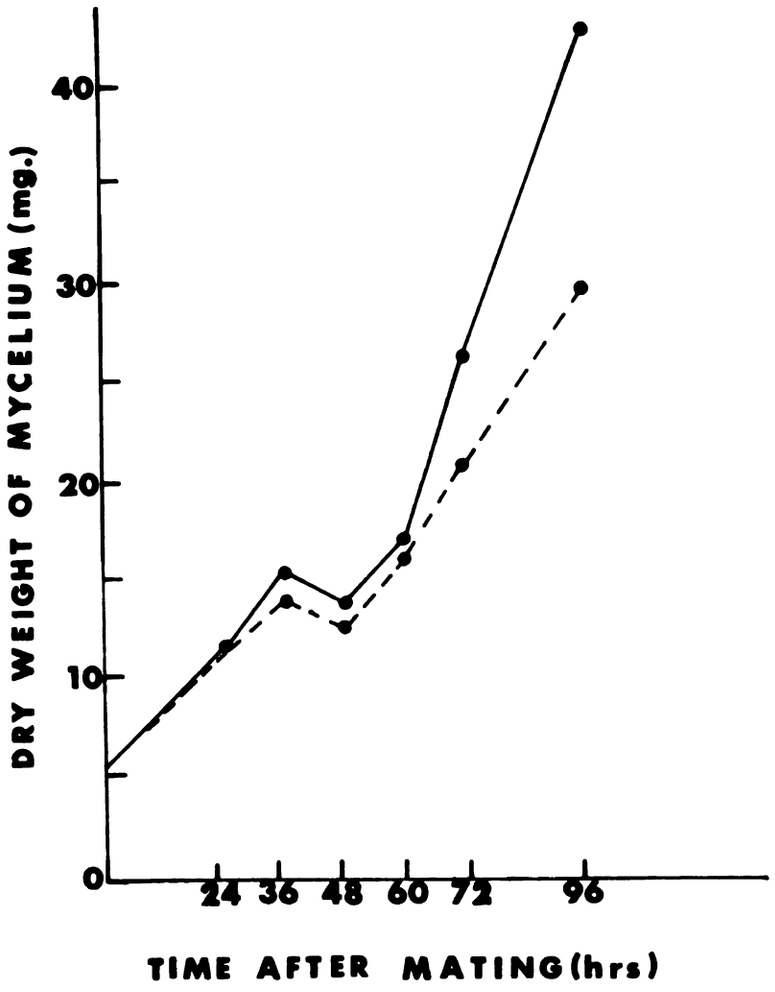


Figure 9. Comparison of growth of a developing dikaryon in complete (●----●) and minimal (●——●) medium.

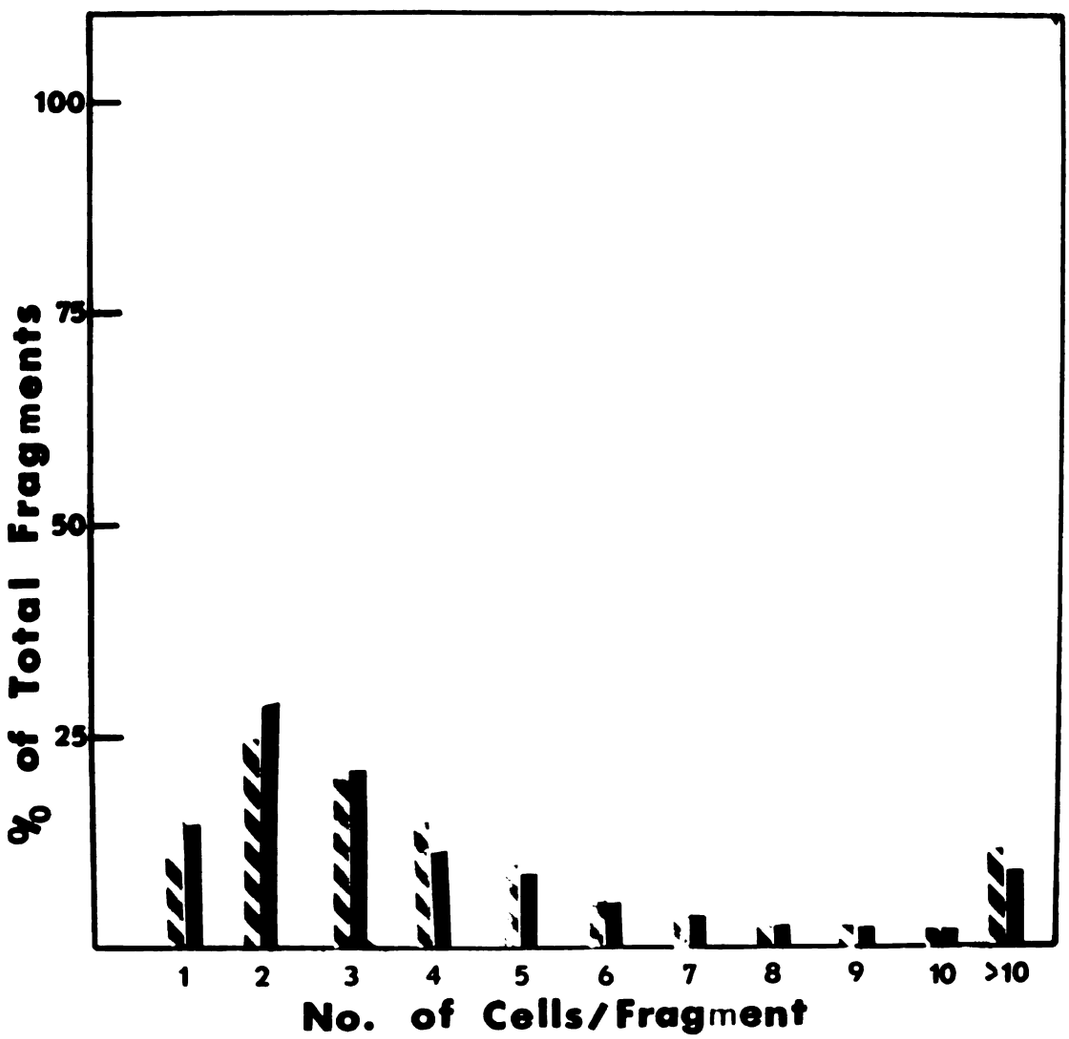


Figure 10. The number of fragments observed with 1-10 cells prior to mating (▨) and after 48 hr of mating (■) in a fully compatible mating.

percentage of fragments so broken as to be devoid of septa which is not presented.

Ribosomes from Vegetative Mycelia

A prime requisite for the studies of cytoplasmic exchange between mating partners was the ability to isolate ribosomes from the mycelia of Schizophyllum commune. In addition, the isolated ribosomes had to be in sufficient amounts for the experimental analyses, and it had to be demonstrated that functional ribosomes and polysomes capable of protein synthetic activity could be obtained.

The method used in the earliest attempts to isolate ribosomes from Schizophyllum was to grind the fresh mycelium in a Tenbroeck tissue homogenizer. This method achieved breakage of a fair number of the hyphal cells but required a very long period of grinding. Even though all the grinding was performed in the cold, sucrose density-gradient centrifugation analysis of the 105,000 X g pellet consistently gave the absorbancy profile shown in Figure 11A. When compared to reticulocyte ribosomes centrifuged as a control sample, and shown in Figure 11B, it can be seen that the absorbancy peaks are in a position higher in the gradient than the reticulocyte monosomes. When the approximate sedimentation coefficients (S values) are computed by the method of Martin and Ames (1961) the major peaks of the Schizophyllum material represent the 60S and 40S ribosomal subunits. Similar results were consistently obtained when fresh or frozen mycelium was used as a ribosome source. The prolonged grinding time apparently results in dissociation of the majority of the ribosomes, possibly because of ribonuclease action.

The finding that large yields of mycelial protein could be obtained from lyophilized mycelium which was ground dry (Ellingboe,

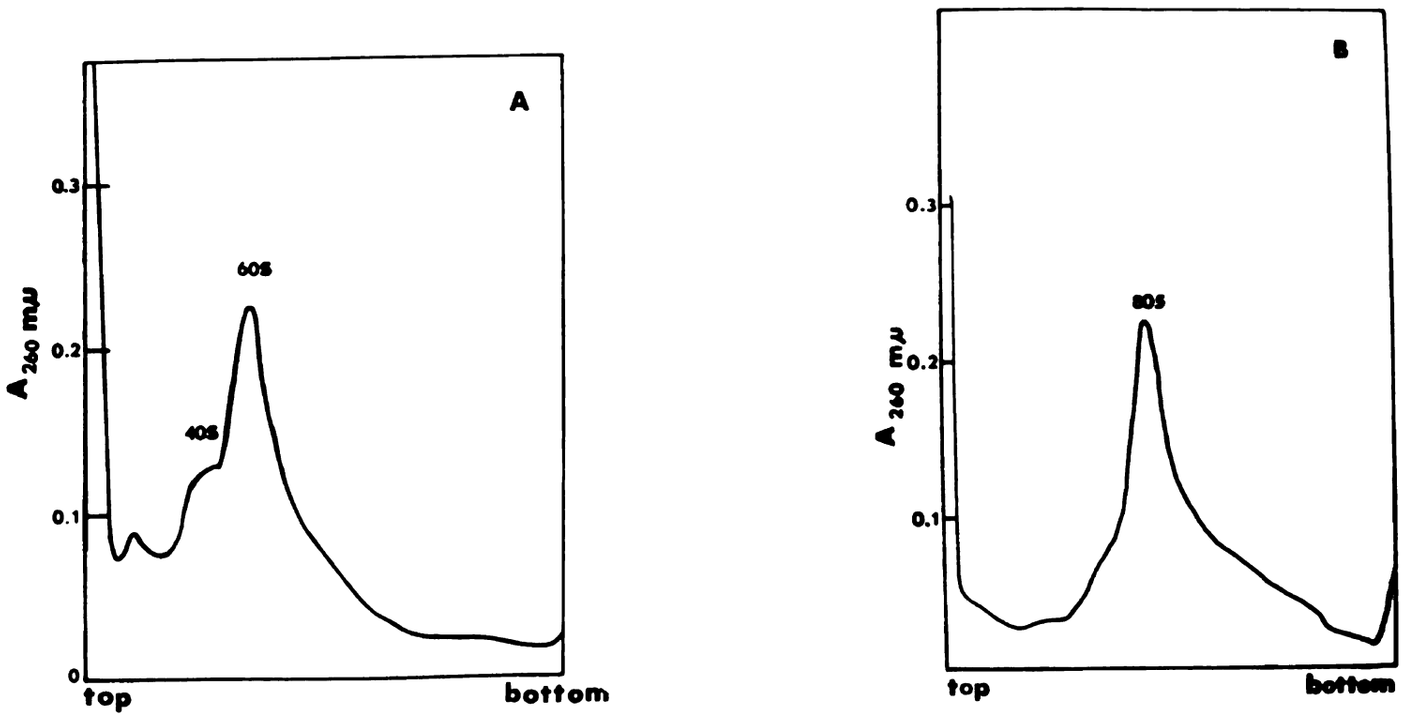


Figure 11. Sucrose density-gradient profiles of ribosomes prepared from (A) Schizophyllum commune mycelium ground fresh and (B) rabbit reticulocytes. Profiles are linear 5-20% gradients.

unpublished results) suggested that many of the problems encountered in previous attempts to isolate ribosomes might be circumvented by using lyophilized mycelium. This technique permits breakage of 100% of the cells.

Sucrose density-gradient absorbancy profiles of ribosomes isolated from lyophilized vegetative mycelia of Schizophyllum and Coprinus are shown in Figure 12A and B, respectively. By comparison with reticulocyte ribosomes centrifuged at the same time, the predominant peak was presumed to consist of monosomes with an approximate S value of 80. The single ribosomes were consistently the major species present in the ribosomal samples prepared from lyophilized mycelia. However, it can be seen that there are classes of ribosomes sedimenting faster than the monosomes. From their position in the gradient in comparison to the reticulocyte ribosome control and the calculated S values, the minor peaks can be considered to represent dimers and trimers.

It was possible that the isolation procedures resulted in artificial aggregation of monosomes and that the heavier classes of ribosomes were the result of such aggregation. To determine whether the Mg^{+2} concentration was possibly responsible for artificial aggregation separate samples of the same Schizophyllum material represented in Figure 12A were prepared in low magnesium buffer (10^{-3}M $MgCl_2$) and low magnesium buffer followed by 30 min treatment in 10^{-3}M EDTA. As seen in Figure 13A and B, although there is some increase in material sedimenting slower than the monosomes and presumed to be ribosomal subunits, the dimers and trimers are still evident. These results indicated that the dimers and trimers represent true polysomal material. Thus, it was clear that at least small numbers of polysomes could be

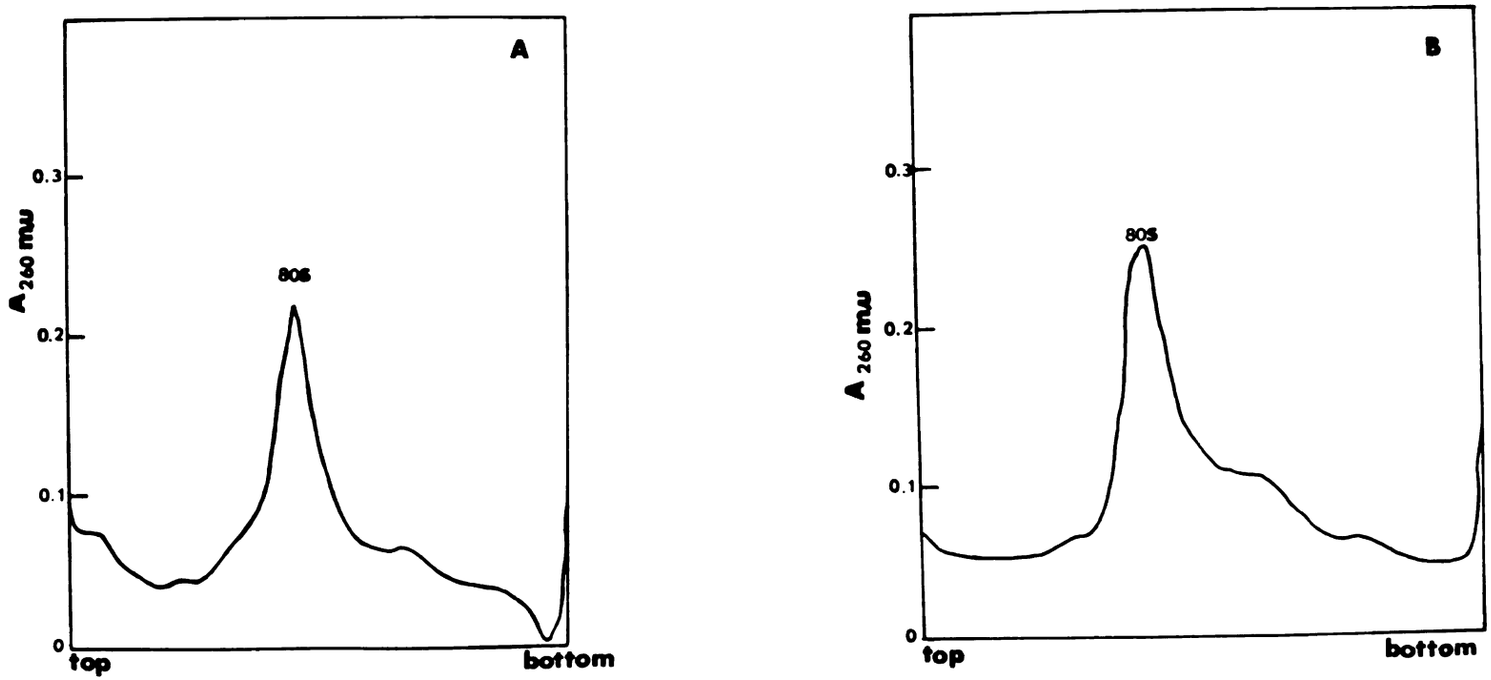


Figure 12. Sucrose density-gradient profiles of ribosomes from vegetative mycelia. Ribosomes were prepared from (A) Schizophyllum commune lyophilized mycelium and (B) Coprinus lagopus lyophilized mycelium. Profiles are from linear 15-30% gradients.

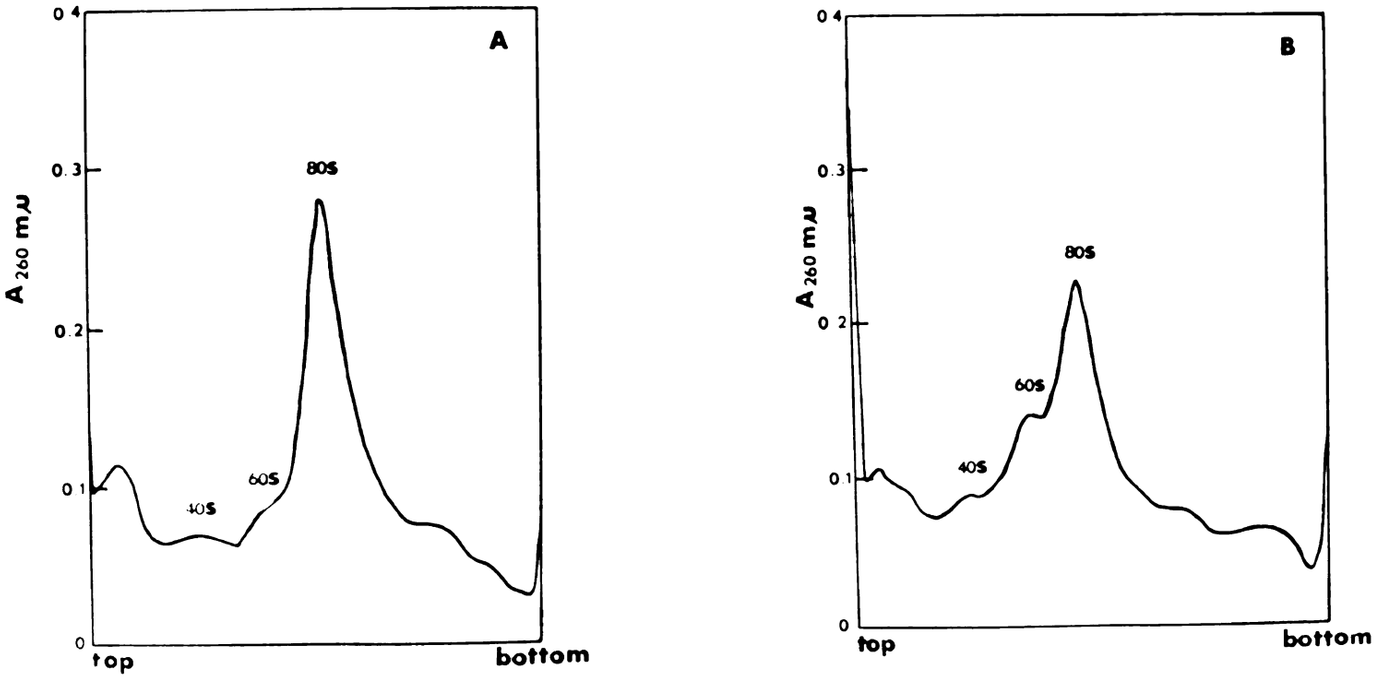


Figure 13. Sucrose density-gradient profiles of ribosomes from Schizophyllum lyophilized mycelium prepared in (A) 10^{-3}M $MgCl_2$ and in (B) 10^{-3}M $MgCl_2$ and resuspended in 2% sucrose + 10^{-3}M EDTA prior to loading on linear 5-20% gradients.

isolated from lyophilized vegetative mycelia by the procedures used.

A good number of ribosomes are bound to the rough endoplasmic reticulum in eukaryotic organisms. Treatment of the crude extract from cells with the detergent sodium deoxycholate (DOC) has been shown to release the ribosomes from the endoplasmic reticulum (Wust and Novelli, 1964). To determine whether DOC treatment resulted in any increase in polysomes, Coprinus mycelium was resuspended in Tris-Mg^{+2} buffer with and without 1% DOC. The ribosomes isolated from the material treated in this way were subjected to sucrose density-gradient centrifugation and the absorbancy profiles are shown in Figure 14A and B. These data indicate that there is some increase in the material sedimenting faster than the monosomes as well as an increase in the 80S monosomes. Therefore, for all subsequent experiments, the ground mycelium was resuspended in Tris-Mg^{+2} buffer containing 1% DOC.

Ribosomes From Ungerminated and Germinating Spores

Germinating spores carry on very active metabolism, including rapid protein synthesis (Sussman and Halvorson, 1966). It was thought that spores and germlings might provide a good source of polysomes. Preliminary experiments had shown that Schizophyllum basidiospores could be obtained in large amounts by the methods described and that these spores could be germinated readily in liquid shake culture. Swelling of the spores occurred after 2-3 hr in supplemented minimal medium and by the sixth hour 70-75% of the spores had produced short germ-tubes. Subsequent development of the germlings consisted of elongation of the germ-tubes. It appeared that the majority of the spores which would germinate did so by the sixth hour and germination never exceeded 80% after 24 hr in culture. Growth of the germlings was not synchronous and whenever samples were examined microscopically,

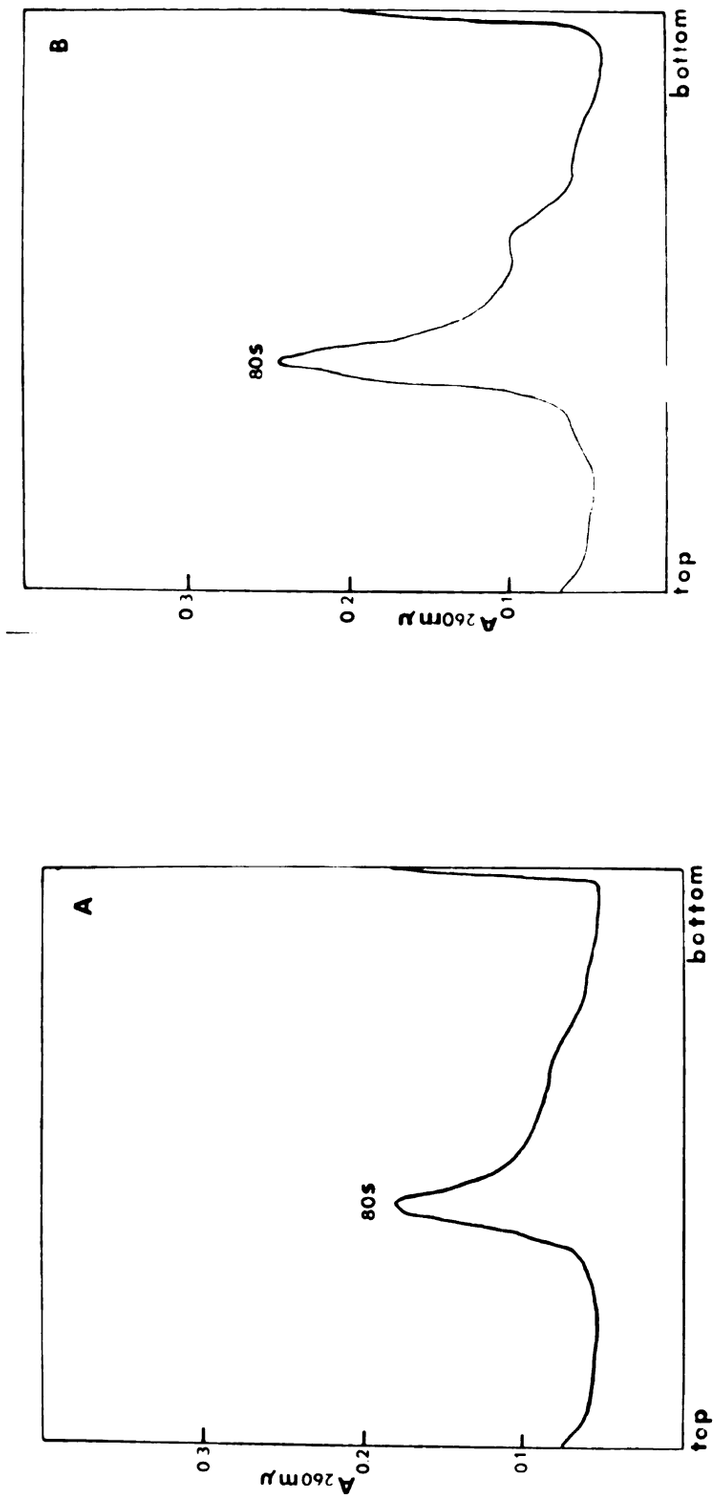


Figure 14. Sucrose density-gradient profiles of ribosomes from Coarctus precared (A) without 1% DOC and (B) with 1% DOC and centrifuged on linear 5-25% gradients.

germ-tubes were of varying lengths.

Experiments were conducted to determine whether the germinating spores would incorporate radioactive amino acid taken up from the medium. The spores used in these experiments were obtained from a mating between an arginine-requiring homokaryon (arg-6) and a nicotinic acid-requiring homokaryon (nic-3). The radioactivity remaining in 1 ml samples of the culture medium supernatant after removal of the germlings by centrifugation at 10,000 X g for 5 min is shown in Figure 15. By the time the spores showed obvious swelling (2-3 hr), less than half the radioactivity remained in the culture medium. At the fifth hour of incubation, approximately 85% of the radioactive arginine had been removed from the medium. Uptake was negligible from 5-15 hrs after inoculation into the radioactive medium. The localization of the radioactivity in the 15,000 X g mycelial pellet and the 105,000 X g supernatant from the germlings harvested at 6, 9, 12 and 15 hrs is given in Table 1. The radioactive arginine incorporated into mycelial structures is at a maximum at 6 hr and decreases from that point onward. The data for the amount of label in the TCA soluble fraction are similar. The ^{14}C-arginine incorporated into TCA precipitable material increases as the radioactivity in the TCA soluble fraction decreases. These data would indicate that the germinating spores remove radioactive amino acid from the medium rapidly and that an appreciable amount is being incorporated into protein. Such spores and germlings should therefore provide a good source of polysomes demonstrable by both absorbancy profiles and radioactivity measurements. Experiments were performed to test this assumption.

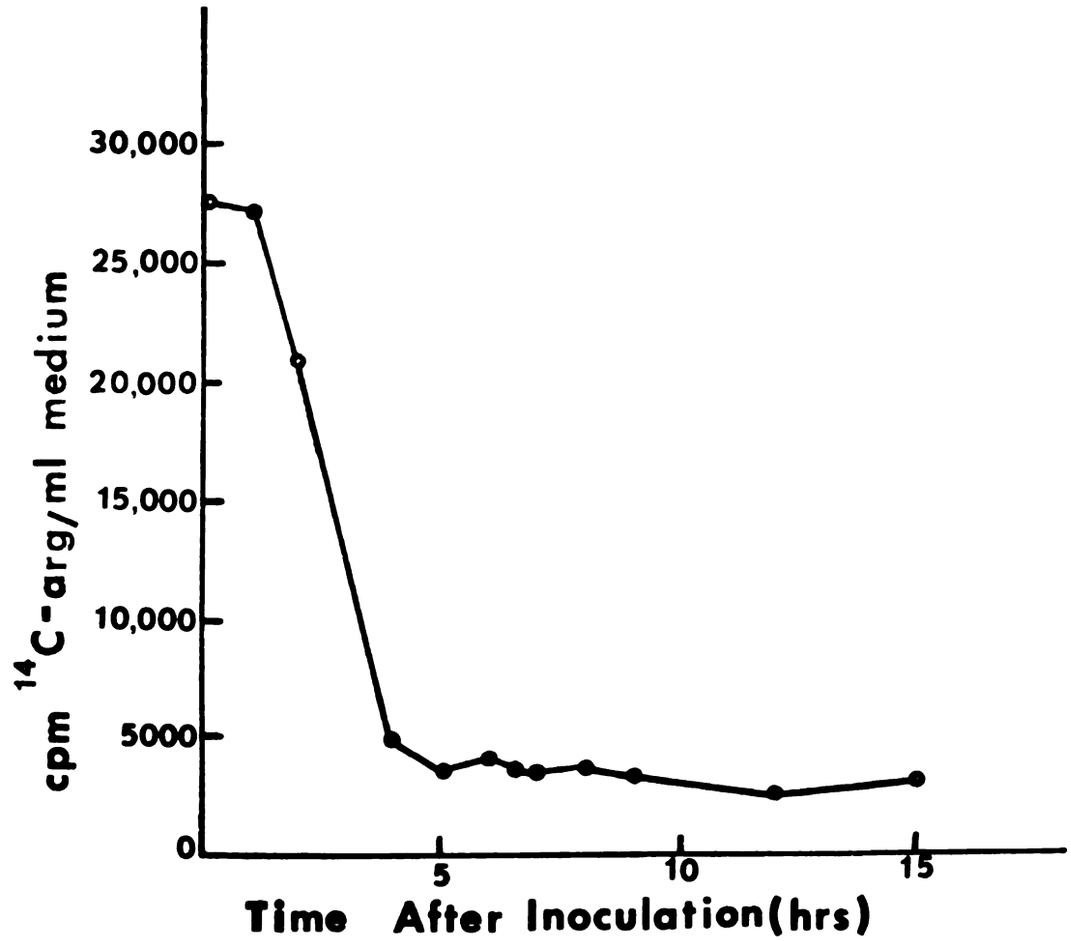
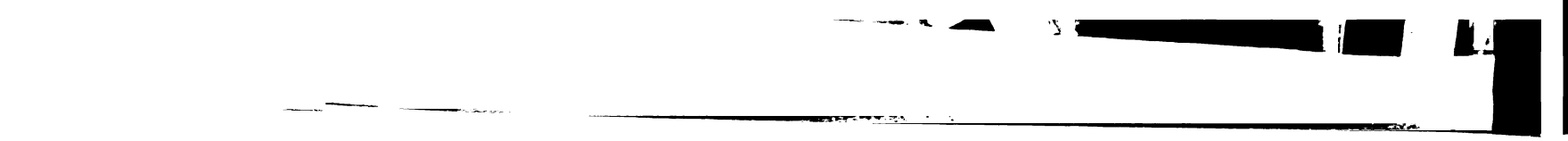


Figure 15. Radioactivity of 1 ml samples of culture medium in which Schizophyllum spores were germinated.

Table 1. Radioactivity in fractions of germinating spore mycelium

Fraction Assayed	Avg cpm ^{14}C-arginine 1 hr	6 hr	9 hr	12 hr	15 hr
15,000 X g pellet (Total mycelial pellet)	108,000	480,000	415,000	375,000	298,000
105,000 X g supernatant (1 ml assayed)					
TCA soluble	75,140/ml	167,350/ml	94,210/ml	91,140/ml	65,720/ml
TCA precipitable	18,745/ml	39,623/ml	37,314/ml	75,391/ml	76,180/ml



Ribosomes isolated from 0 hr spores and 6, 9, and 12 hr germlings gave distinctive absorbancy profiles. In the 0 hr spores (Figure 16), the monosomes are the predominant species present but there is evidence for some heavier classes of polysomes. The 1 hr germlings (Figure 17A) yield very little ribosomal material and the absorbance peak is at a point in the gradient corresponding to that of ribosomal subunits. The absorbancy profile of ribosomes from 6 hr germlings (Figure 18A) provides good evidence that at least one additional class of polysomes can be resolved under the conditions used. Results similar to those from 6 hr germlings were also obtained for 9 and 12 hr germlings and are shown in Figure 19A and 20A respectively. The correspondence between the absorbancy and radioactivity is shown for 1, 6, 9 and 12 hr germlings in Figures 17B, 18B, 19B and 20B respectively.

Further evidence that material sedimenting faster than the monosomes actually does represent various classes of polysomes was obtained from experiments in which the ribosomes were treated with pancreatic ribonuclease prior to sucrose density-gradient centrifugation. The ground mycelium from 15 hr germlings was divided into two portions and the ribosomes isolated from both simultaneously. One ribosomal pellet was resuspended in 2% sucrose while the other was resuspended in 2% sucrose containing 4 μg ribonuclease. Both samples were allowed to stand for 90 min in the cold (4^{o}C) and then layered on 5-20% linear sucrose gradients and centrifuged. From the results shown in Figure 21, it can be seen that the ribonuclease treatment clearly results in the movement of the majority of the radioactivity into the monosome peak and a considerable amount into the subunit region of the gradient.

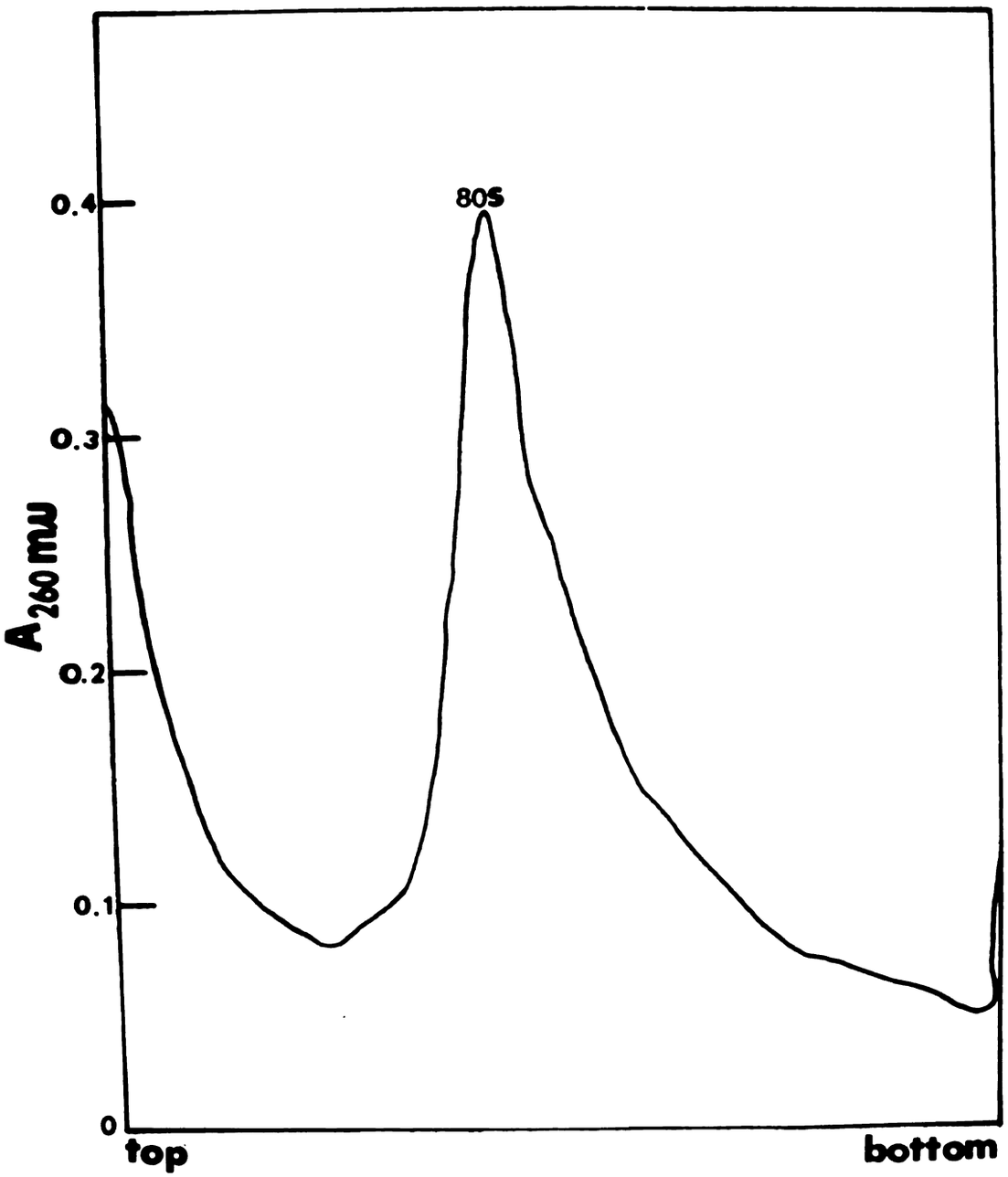
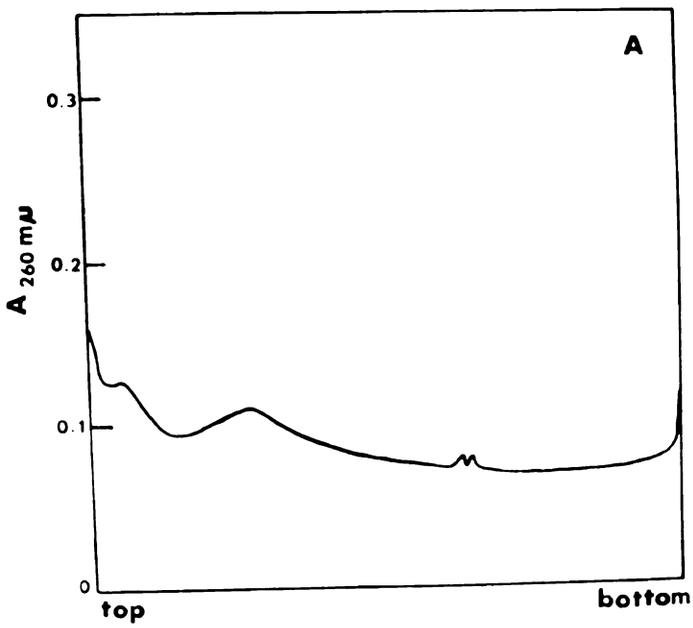


Figure 16. Sucrose density-gradient profile of ribosomes from ungerminated (0 hr) Schizophyllum spores. 15-3% linear gradients were used.



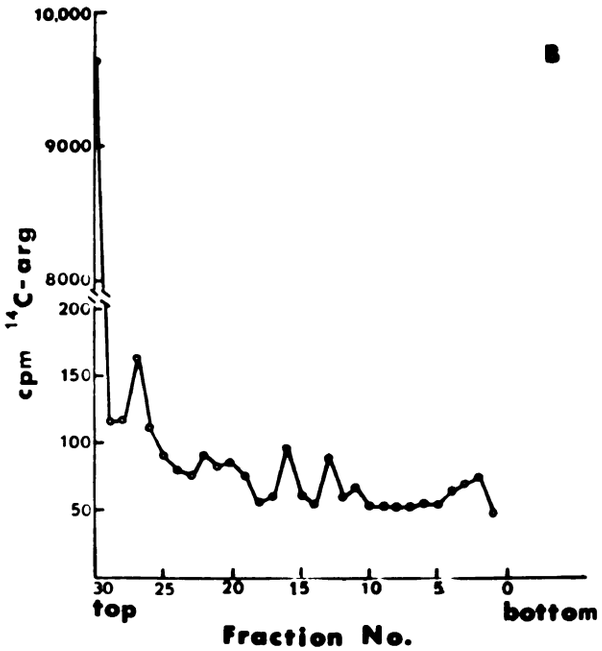


Figure 17. Sucrose density-gradient profiles of 1 hr germlings of Schizophyllum. In (A) the absorbancy profile is shown and in (B) the radioactivity profile is shown. 15-37% linear gradients were used.

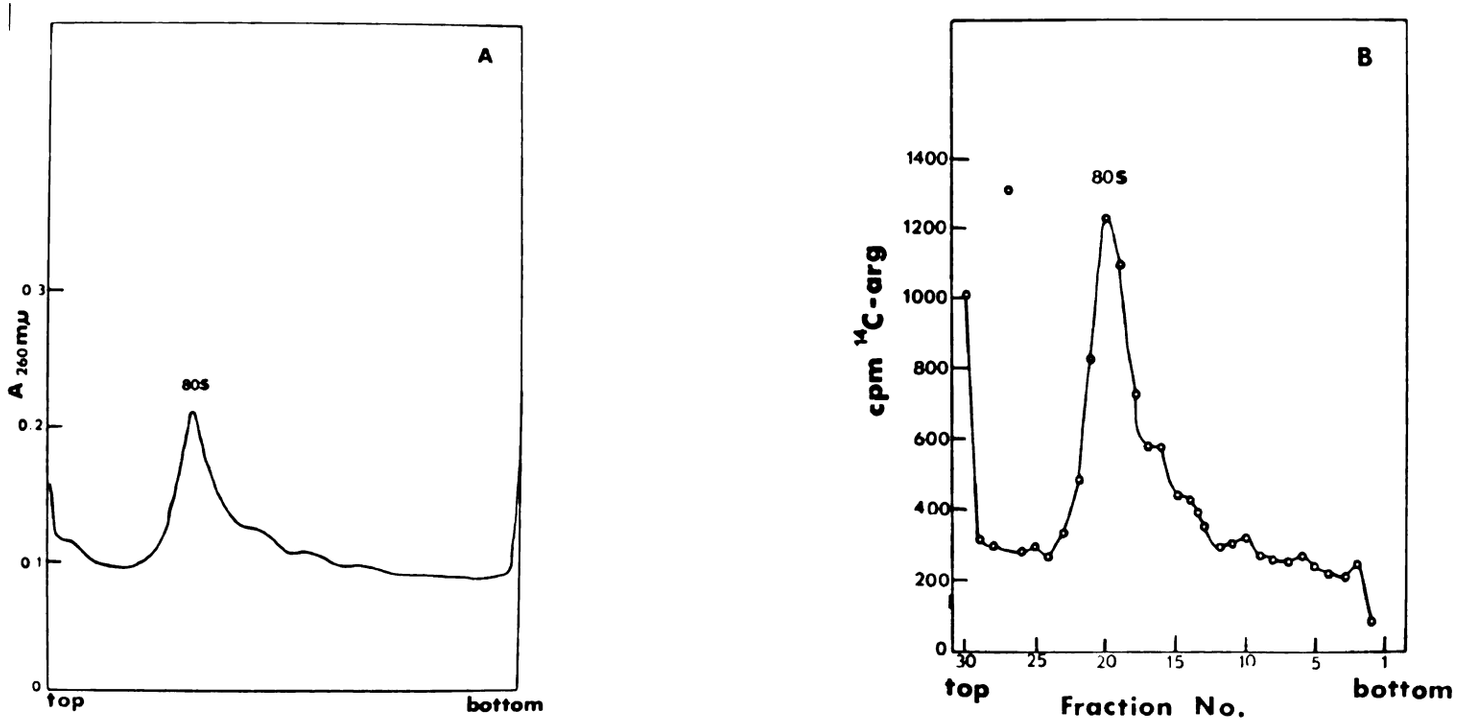


Figure 18. Sucrose density-gradient profiles of 6 hr germlings of Schizophyllum. In (A) the absorbancy profile is shown and in (B) the radioactivity profile is shown. 15-30% linear gradients were used.

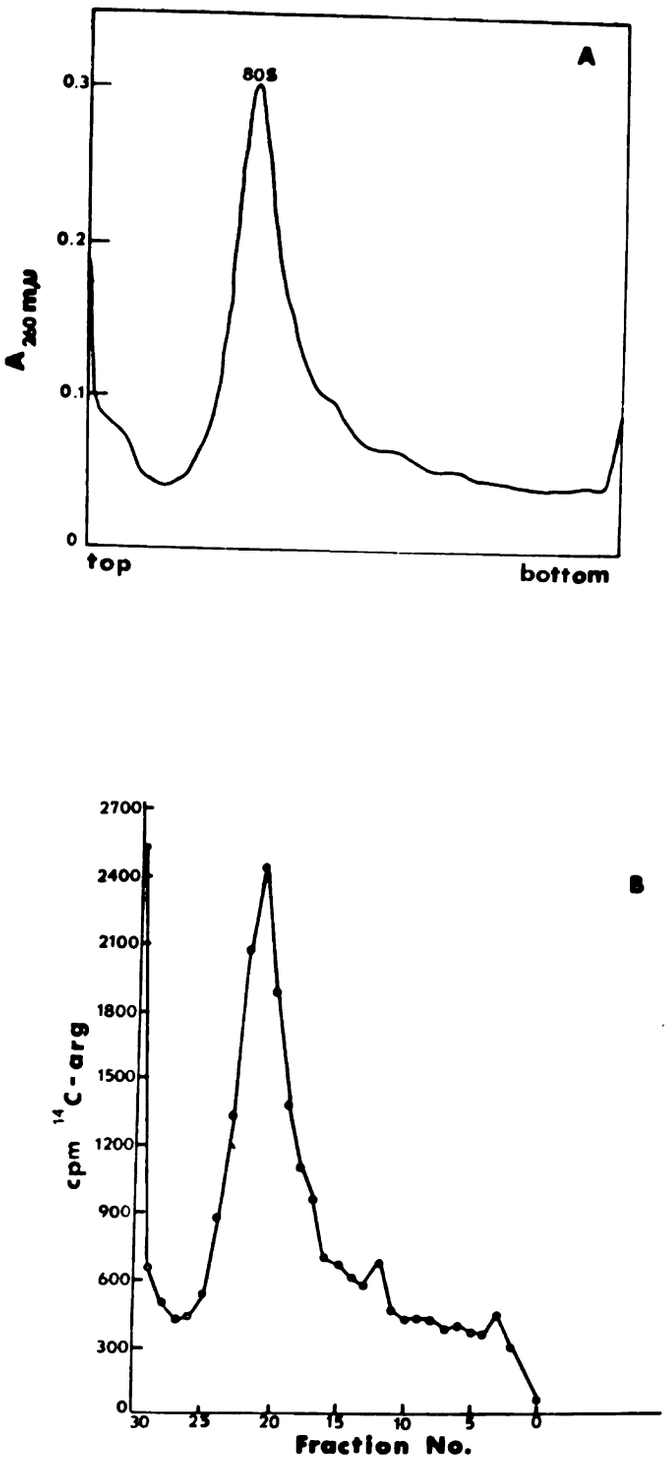
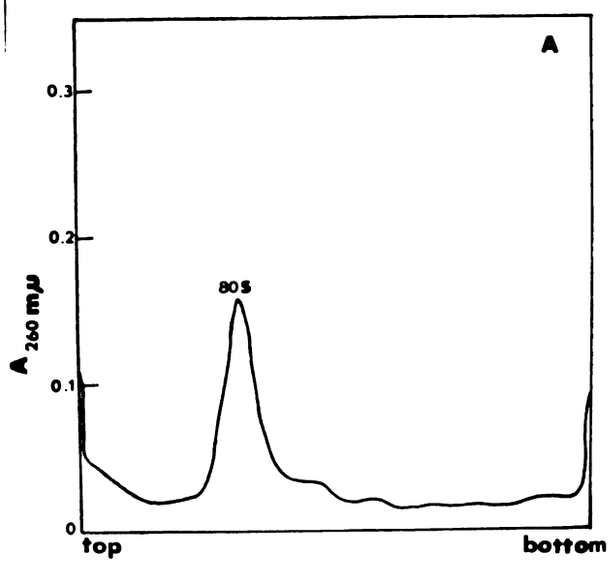


Figure 19. Sucrose density-gradient profiles of 9 hr germlings of Schizophyllum. In (A) represents the absorbancy profile and in (B) the radioactivity profile is shown. 15-30% linear gradients were used.



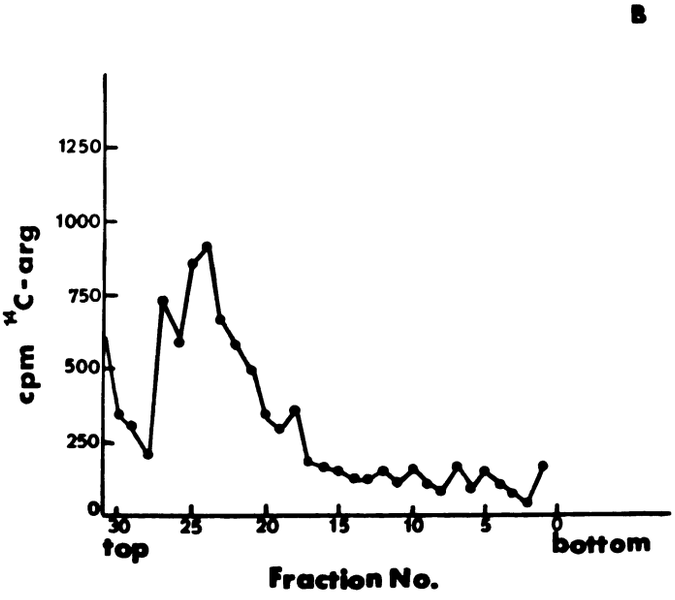


Figure 20. Sucrose density-gradient profiles of 12 hr germlings of Schizophyllum. In (A) the absorbancy profile is shown and in (B) the radioactivity profile is shown. 15-30% linear gradients were used.

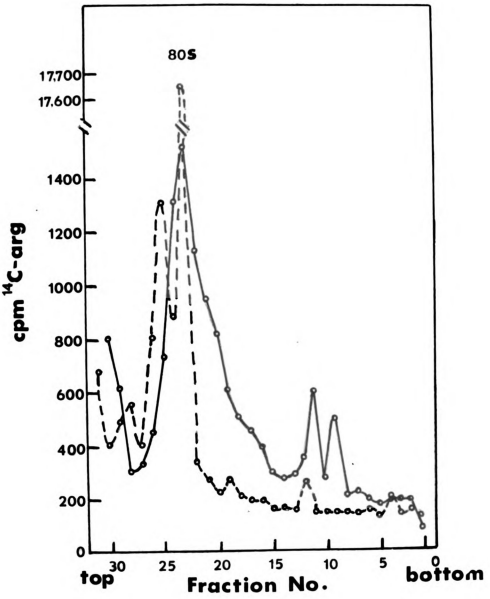


Figure 21. The effect of ribonuclease treatment on the polysomes from 15 hr Schizophyllum germlings. The ribosomes were prepared from separate samples of the same lyophilized material. One ribosomal pellet was resuspended in 2% sucrose, the other in 2% sucrose containing 4 μg per ml pancreatic ribonuclease. Both samples were allowed to stand for 90 min in the cold (4°C). Each sample was then analyzed on a linear 15-30% sucrose density gradient. (O——O=untreated; O----O=RNAase-treated)

These results, taken together with those obtained with ribosomes from vegetative mycelia, indicate that intact monosomes and small populations of various classes of polysomes are isolatable from lyophilized material which has been subjected to prolonged grinding with glass beads in the dry state.

Amino-Acid Uptake in a Cell-Free System

Although the results from sucrose density-gradient analysis of the isolated ribosomes offer evidence that monosomes and polysomes could be isolated from lyophilized fungal material, evidence was needed that the ribosomes were not considerably altered by the lyophilization and grinding procedures. If the ribosomes were not in the native state and capable of involvement in protein synthesis, then these procedures would be of little value in subsequent studies on cytoplasmic exchange. Therefore, it was considered necessary to demonstrate the functional nature of the ribosomes by assaying their ability to incorporate amino-acids into TCA precipitable material in a cell-free system. In addition, by excluding exogenous messenger RNA (mRNA), the functional capacity of the mRNA contained with the polysomes could be ascertained. The cell-free system developed for the study of hemoglobin synthesis by reticulocyte ribosomes was used. The fungal ribosomes were substituted for reticulocyte ribosomes and all other components were obtained from rabbit reticulocytes. In this way the ability of the fungal ribosomes to direct amino acid incorporation could be measured directly without possible confusion by other components of the system. Also the activity of the fungal ribosomes could be compared to that of reticulocyte ribosomes. The results of these experiments are compiled in Table 2. From these data, it can be seen that ribosomes from

Table 2. Amino acid incorporation by fungal ribosomes in a cell-free system.

The complete system is as described in Materials and Methods. The concentrations of ribosomes were based on the extinction coefficient of 11.3 A_{260} units/mg reticulocyte ribosomal RNA

Ribosome Source	Amount of Ribosomes/ml incubation mixture	^{14}C-valine incorporated	
		cpm	μμM
Control*	---	149	3
Coprinus homokaryon	0.2 mg	418	11
	0.5 mg	672	18
Coprinus dikaryon	0.2 mg	352	9
	0.5 mg	543	14
	1.0 mg	1065	29
Schizophyllum homokaryon	0.2 mg	424	11
	0.4 mg	847	23
0 hr spores	0.25 mg	1137	31
1 hr spores	0.5 mg	748	20
6 hr spores	0.2 mg	662	18
	0.4 mg	1097	30
reticulocyte	0.5 mg	7116	202

* Control = complete system without ribosomes

Schizophyllum and Coprinus vegetative mycelia and from Schizophyllum spores and germlings are functional in in vitro protein synthesis. The amount of amino-acid incorporated is shown to be proportional to the concentration of ribosomes. Since no exogenous mRNA is added, this proportionality would indicate that the capacity for amino-acid incorporation is governed by the amount of polysomes and endogenous mRNA present. This is further supported by the comparison of the data for 1 hr spores with those for the 0 hr and 6 hr spores. The 1 hr spores consistently yields few polysomes and mostly ribosome subunits. Therefore, the amount of mRNA present is low and less amino-acid incorporation is possible. The fungal ribosomes are about 10-15% as efficient as reticulocyte ribosomes in this system. This is further evidence that the amount of mRNA present is an important factor since the reticulocyte 1X ribosomes used in these experiments were known to consist largely of polysomes. The lower efficiency is also possibly due to the specificity of the enzymes and transfer RNA, all of which are derived from the reticulocyte hemoglobin synthesizing system. Another possible explanation for the lower efficiency is that the fungal ribosomes are damaged by the lyophilization and grinding and only those ribosomes not damaged are functional. The possibility that there is an inhibitor present on the Schizophyllum and Coprinus ribosomes which lowers their efficiency in a foreign system also exists. However, such an inhibitor should affect the proportionality of amino-acid incorporation vs. ribosome concentration, and it does not.

Growth of Homokaryon in Deuterium-Containing Medium

In order to obtain ribosomes from one homokaryotic strain which could be separated from the ribosomes from another homokaryontic strain,

it was decided to differentially label the strains by growing one in deuterium (D_2O) medium and the other in medium containing ^{14}C-arginine. It has been reported that some fungi grow poorly in D_2O medium (Henderson and Lamonds, 1966). The effect of D_2O medium on the growth of wild-type homokaryon 699 was determined. Two separate experiments were conducted in which the dry weight of the mycelium and the dry weight of the polysaccharide produced were measured after growth for 5 days in medium having different concentrations of D_2O. The averaged results are presented in Figure 22. In A, the dry weight of the mycelium is compared to the percentage of D_2O used in place of H_2O. In 100% D_2O-medium, the dry weight of the mycelium is nearly equal to that in regular minimal medium. The measurement of the dry weight of the extracellular polysaccharide produced in the various concentrations of D_2O (B) gives similar results. It can be seen that the homokaryon can be grown successfully in D_2O-medium. Also, the amount of mycelium produced in D_2O should be nearly equal to the amount of the other homokaryon to be used in the mating. Utilization of the D_2O in growth should insure that the density of the ribosomes would be sufficiently increased to make them separable from the ribosomes from another strain grown in H_2O-medium.

Equilibrium Density-Gradient Centrifugation

Preliminary experiments were conducted to determine the conditions most favorable for analysis of cytoplasmic exchange by separation of D_2O-labelled ribosomes and those from the mycelium grown in H_2O. To establish these conditions, 1X ribosomes isolated from Schizophyllum homokaryons and dikaryons grown in H_2O-medium and various Coprinus cultures were subjected to CsCl density-gradient centrifugation. In

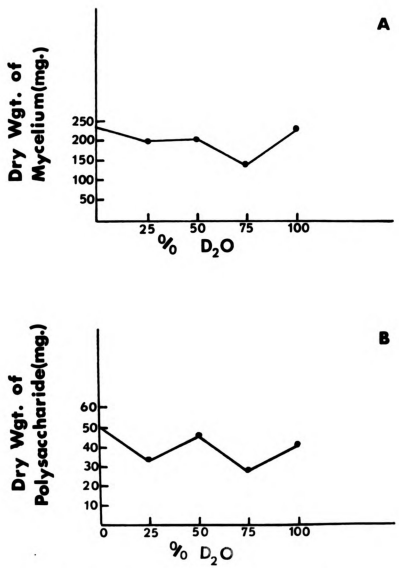


Figure 22. Growth of Schizophyllum homokaryon in medium containing D_2O. In (A) the dry weight of the mycelium is shown and in (B) the dry weight of the polysaccharide is shown.

Figure 23, the location of the bands in typical gradients is shown. The ribosomes from mycelium grown in H_2O (A) reach equilibrium at a point near the top of the gradient whereas those from mycelium grown in D_2O (B) band out considerably lower in the tube. Similar results were always obtained when the light and heavy ribosomes were centrifuged individually. The centrifugation of the ribosomes obtained from a 48 hr mating of Schizophyllum homokaryons in H_2O medium gave the result shown in Figure 23C. The band is located near the top of the gradient in a position similar to that of the homokaryon grown in H_2O. The absorbancy at 260 mμ of fractions from CsCl gradients of ribosomes from such mycelia is shown in Figure 24-26. The absorbancy profile of fractions obtained from a CsCl gradient of ribosomes from Coprinus mycelium is shown in Figure 27. To demonstrate that the light and heavy ribosomes were separable when centrifuged together, 1X ribosomes from cultures grown in H_2O and D_2O were mixed and the mixture centrifuged at 40,000 rpm for 18 hr. The results of two such centrifugations are shown in Figure 28. In A, the mixture consisted of ribosomes from Schizophyllum homokaryons grown in H_2O and D_2O medium, and in B, the mixture consisted of ribosomes from Coprinus homokaryon grown in H_2O and Schizophyllum homokaryon grown in D_2O. These results demonstrate that the density-labelled ribosomes are clearly separable from the light ribosomes when the two classes are mixed artificially. By centrifuging ribosomes from homokaryons grown in H_2O-medium containing ^{14}C-arginine, it was possible to obtain an idea of where the radioactivity would be localized in the gradient. The results most consistently obtained are presented in Figure 29 and Figure 30. When the homokaryon ribosomes

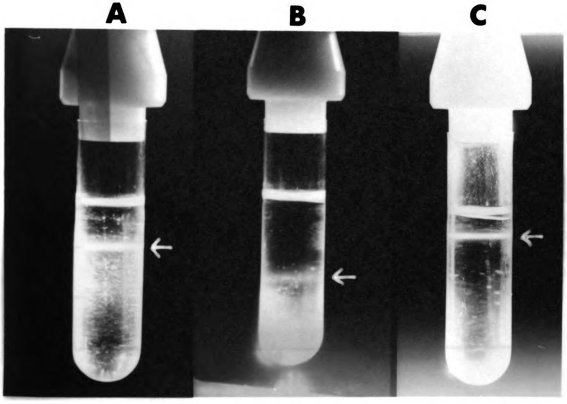


Figure 23. Results of CsCl density-gradient centrifugation of Schizophyllum ribosomes for 18 hr at 40,000 rpm; (A) ribosomes from mycelium grown in H_2O medium, (B) ribosomes from mycelium grown in D_2O medium, and (C) ribosomes from 48 hr mating in H_2O medium.

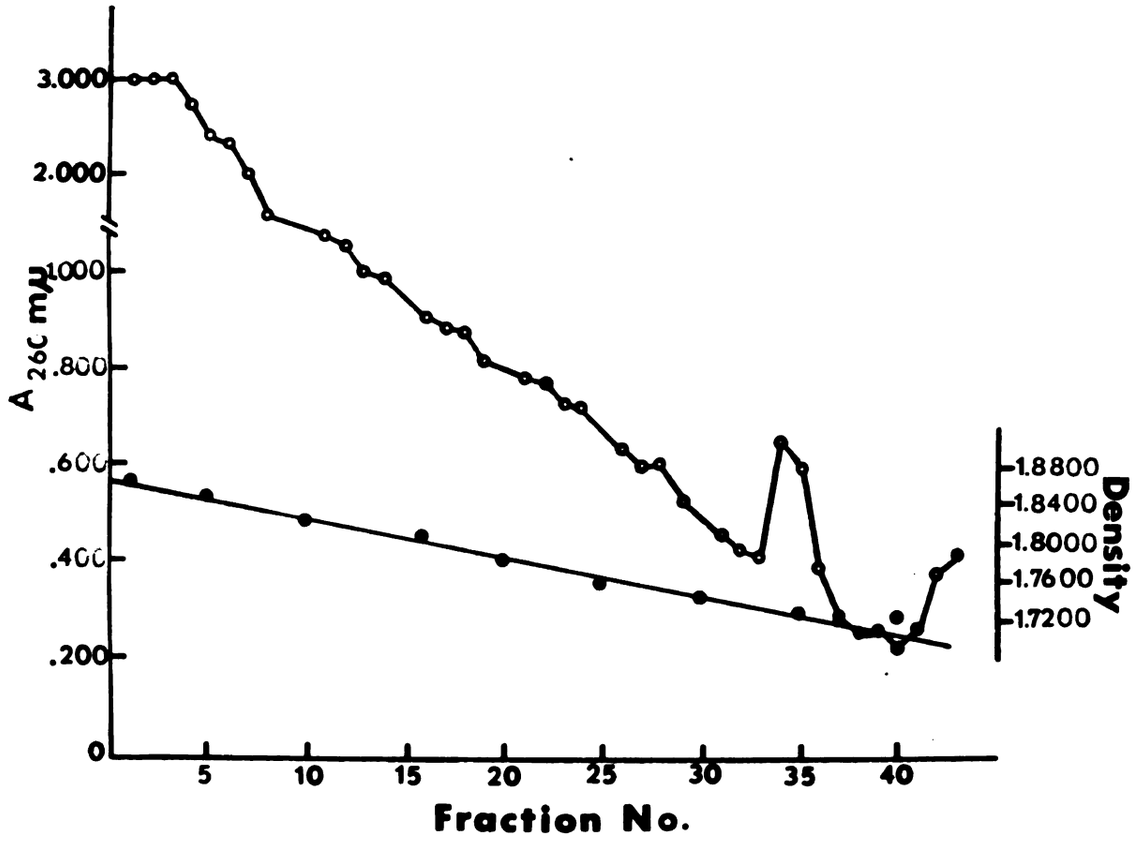


Figure 24. Absorbancy profile of 10-drop fractions obtained from CsCl density-gradients of ribosomes from homokaryon grown in H_2O medium shown in Figure 23(A).

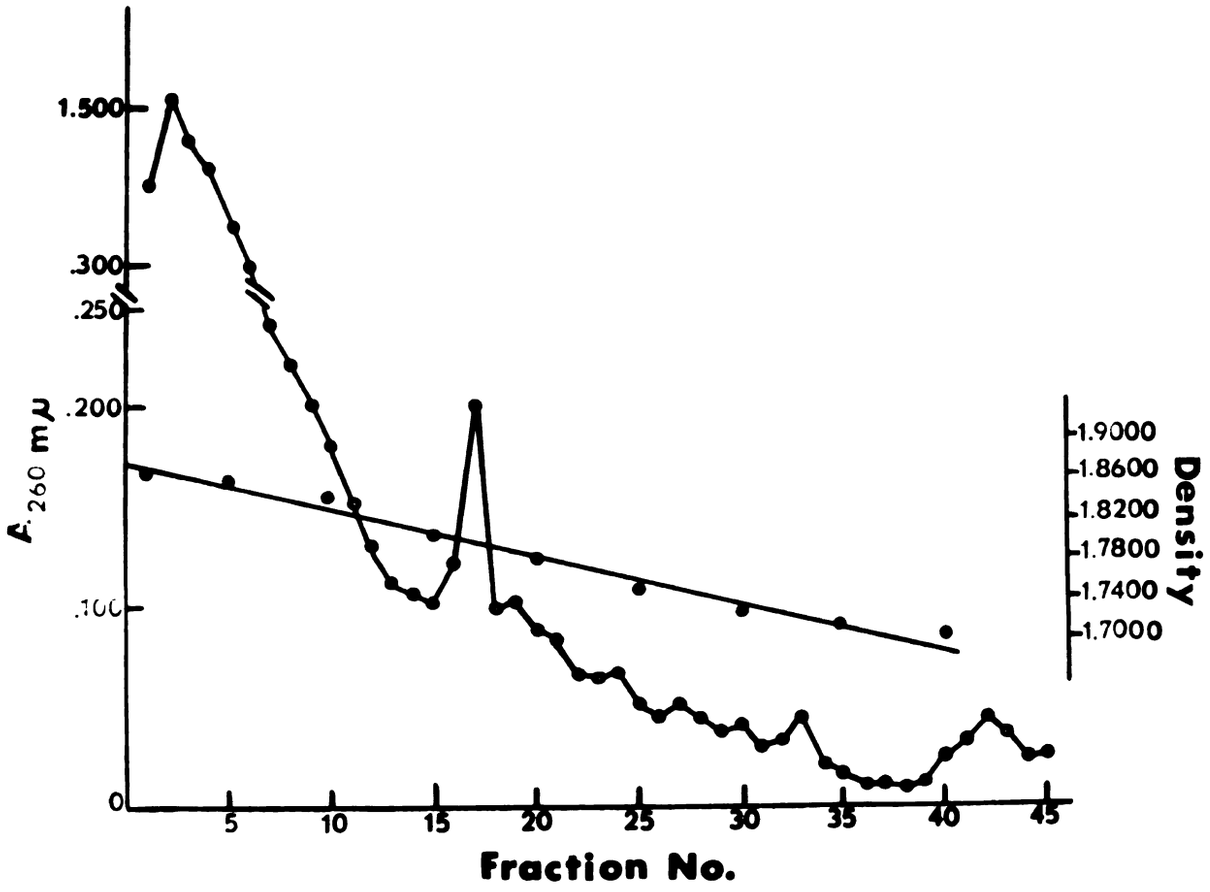


Figure 25. Absorbancy profile of 10-drop fractions obtained from CsCl density-gradient of ribosomes from heterokaryon grown in D_2O medium shown in Figure 23(B).

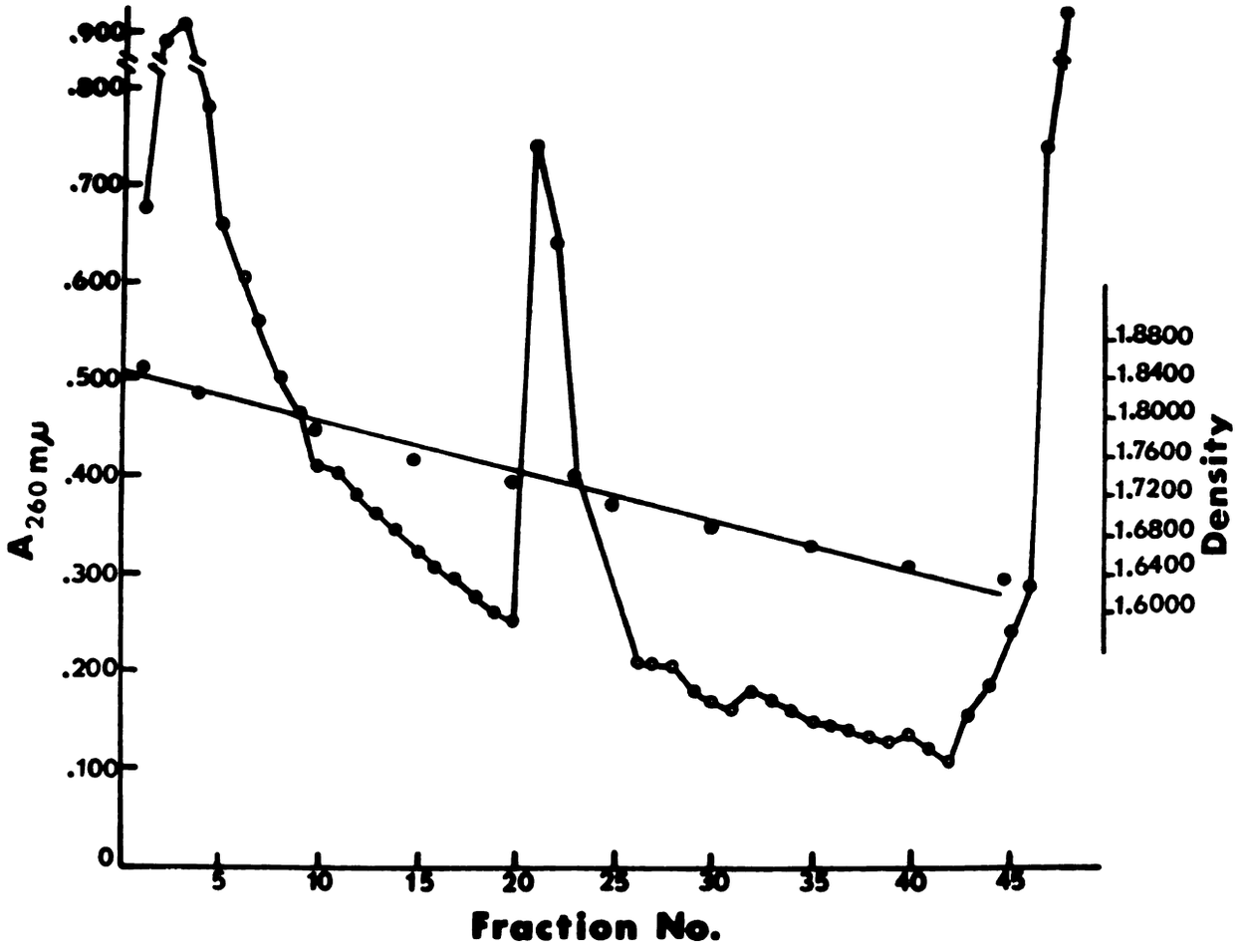


Figure 26. Absorbancy profiles of 10-drop fractions from CsCl density-gradient of ribosomes from 48 hr mating of homokaryons grown in H_2O medium shown in Figure 23(C).

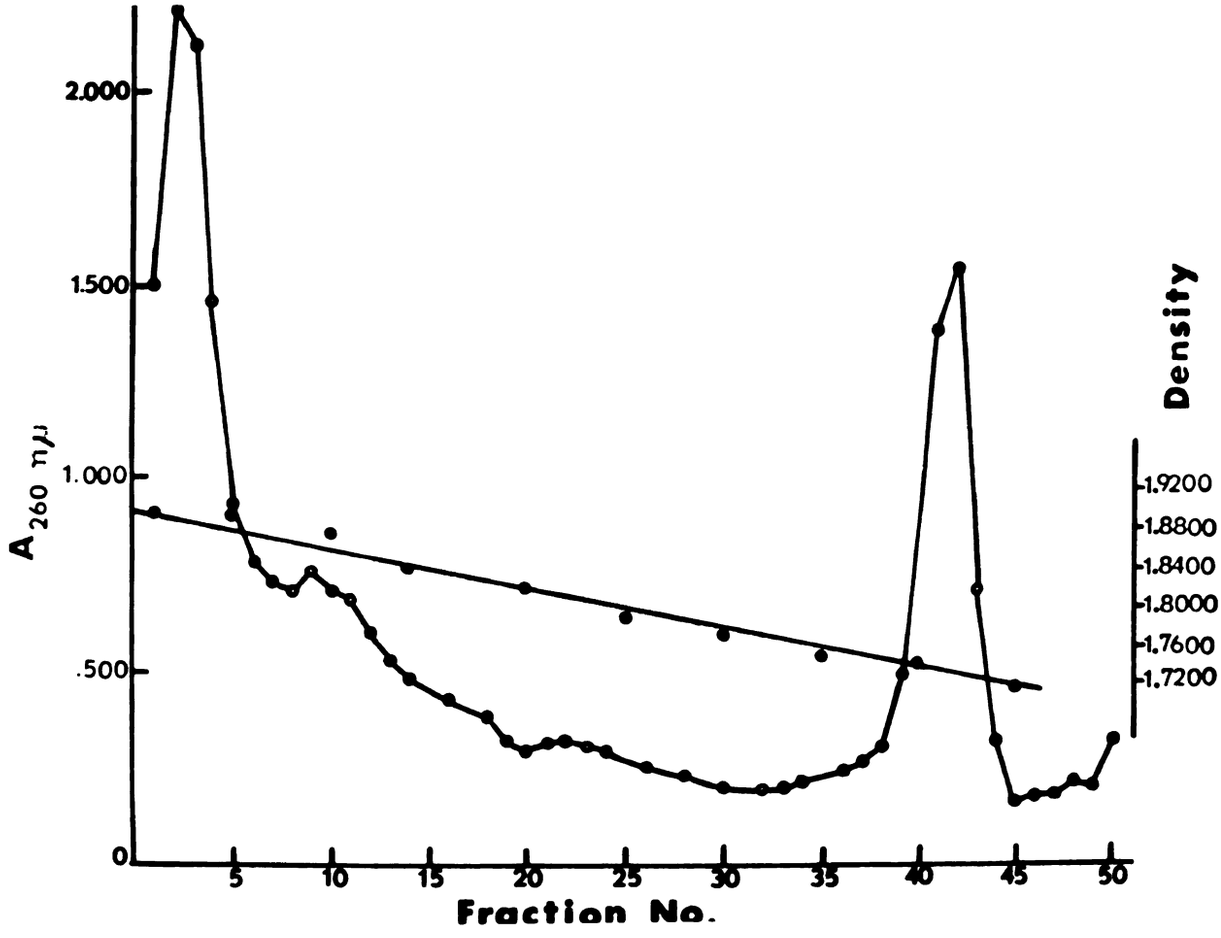


Figure 27. Absorbancy profile of 10-drop fractions from CsCl density-gradient of ribosomes from Coprinus mycelium grown in H_2O medium.

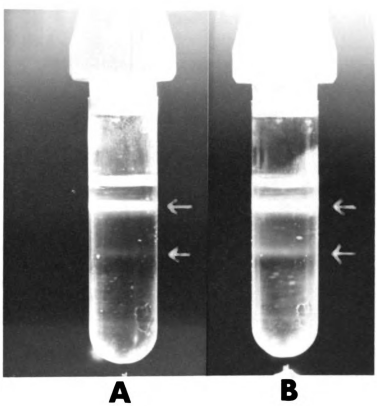


Figure 28. Results of CsCl density-gradient centrifugation of artificial mixtures of ribosomes for 18 hr at 40,000 rpm. (A) represents the mixture of Schizophyllum homokaryons grown in H_2O medium and D_2O medium and (B) represents the mixture of Coprinus mycelium grown in H_2O medium and Schizophyllum mycelium grown in D_2O medium.

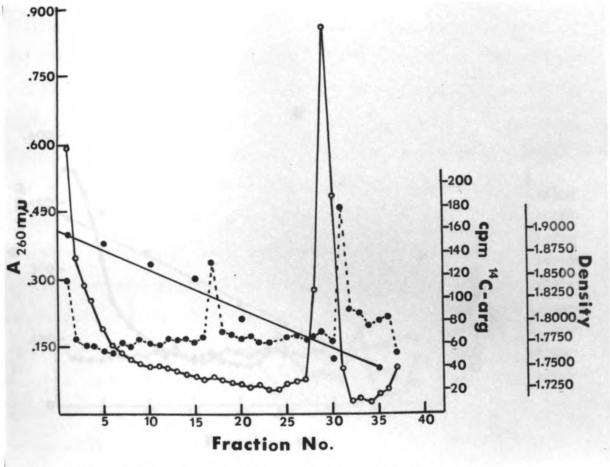


Figure 29. CsCl density-gradient profiles of ribosomes from Schizophyllum homokaryon grown in H_2O-medium containing 2 μc ^{14}C-arginine, (O——O, absorbancy; ●----●, radioactivity).

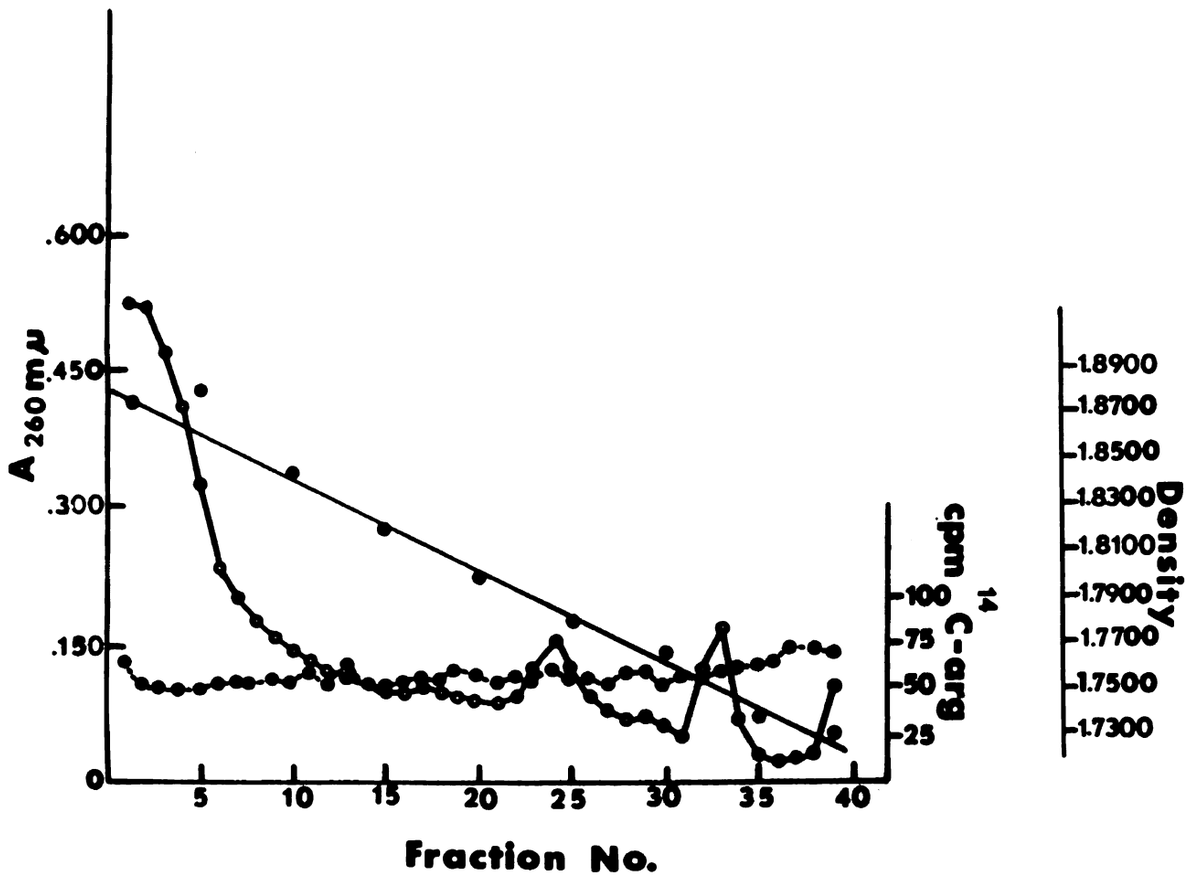


Figure 30. CsCl density-gradient profiles of ribosomes from artificial mixture of Schizophyllum homokaryon grown in H_2O-medium containing 2 μc ^{14}C-arginine and homokaryon grown in D_2O-medium. (O——O, absorbancy; ●----●, radioactivity).

were centrifuged alone (Figure 29), the radioactivity was concentrated in two regions. The minor peak is in a region near the center of the gradient at a calculated density of 1.8250 g/cc. The basic absorbancy pattern is one of a steady decline. The density at which this radioactivity was located is greater than the density at which the heavy and light ribosomes banded. Therefore, it is thought that some other unidentified component is responsible for the radioactivity peak in the heavy region. The major peak of radioactivity is in the same region of the gradient as the absorbancy peak, though the radioactivity maximum is two fractions removed from the absorbancy maximum. There is a slight increase in radioactivity corresponding to the absorbancy peak and the radioactivity increases throughout the region in which there is an increase in absorbancy, fractions 25-31. The reason for the displacement of the radioactivity into a less dense region is not known. Figure 30 represents the results obtained when an artificial mixture of ^{14}C-arginine labelled ribosomes and heavy ribosomes was centrifuged. The amount of radioactivity detected is quite low, but there is an increase in radioactivity throughout the region of the gradient in which the light ribosomes were located. The greatest radioactivity is again near the top of the gradient. These results suggested that it was feasible to proceed with the studies on cytoplasmic exchange, since centrifugal separation of heavy and light ribosomes was possible and random association of ^{14}C-arginine with the heavy ribosomes did not occur to any significant extent.

Localization of Radioactivity in Schizophyllum Homokaryons

The uptake of significant amounts of ^{14}C-arginine by the homokaryon which was to be mated with the homokaryon grown in D_2O was

essential. In addition, it was necessary to know what percentage of the radioactivity was still in the amino-acid pool available for exchange and utilization by the mate. Finally, an indication of the degree of dilution of the radioactivity incorporated should be obtained. To accomplish this, the arginine-requiring homokaryon to be used in the matings was grown in 1 μc ^{14}C-arginine for 24 hrs. After this period, one-half of the flasks was harvested, lyophilized and ground. The other half was macerated, washed, inoculated into supplemented minimal medium without ^{14}C-arginine, incubated in shake culture for 24 hr and harvested, lyophilized and ground. The results of one such experiment in which all fractions were analyzed are presented. In other experiments, a complete analysis was not made but the results from each experiment do agree with similar determinations presented here.

Figure 31 shows the kinetics of disappearance of the label from the medium. Through the first 9 hrs, no detectable quantity of radioactive arginine was removed from the medium. By the 18th hr of incubation, less than 50% of the radioactivity remained in the medium and by the 24th hr, only about 10% remained. This indicates that very significant amounts of radioactive arginine are taken up during growth of the homokaryon. In Table 3, the radioactivity in each fraction of the ground mycelia is presented. In addition, the radioactivity of similar fractions derived from the mycelium after 24 hr growth in unlabelled medium is presented for comparison. These data indicate that a considerable amount of radioactivity is found in each fraction assayed after 24 hr growth. The assay of the aliquots from the mycelium grown an additional 24 hrs in unlabelled medium indicates that the additional growth and synthesis results in dilution of the radioactivity in the

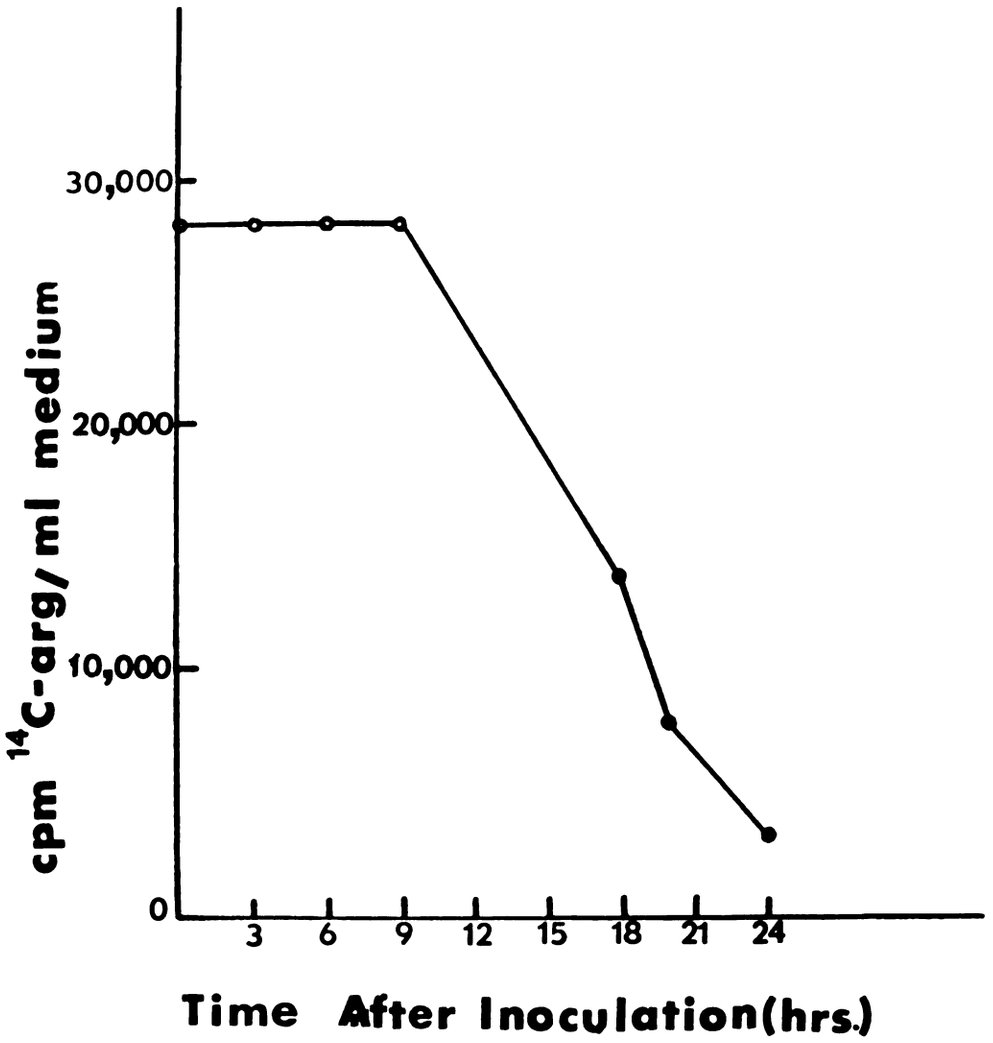


Figure 31. Radioactivity of 1 ml samples of culture medium in which arginine-requiring homokaryon was grown.

Table 3. Radioactivity in fractions of 150 mg of ground homokaryotic mycelium, grown 24 hr in labelled medium and subsequently for 24 hr in unlabelled medium

Fraction Assayed	24 hr 1 μc ^{14}C-arg		24 hr 1 μc ^{14}C-arg + 24 hr unlabelled medium	
	cpm/aliquot	Total	cpm/aliquot	Total
Culture medium	3178/ml	63,560/20 ml	508/ml	20,320/50 ml
Wash supernatant				
1st wash	903/ml	60,900/40 ml	-	-
2nd wash	334/ml	26,700/80 ml	-	-
15,000 X g pellet	1047/mg	157,050/150 mg	694/mg	102,100/150 mg
ribosomes	6016/mg RNA	9,018/1.5 mg RNA	1632/mg RNA	2,668/1.8 mg RNA
9,000 X g pellet from 1X ribosomes	22/mg	3,331/150 mg	12/mg	1,951/150 mg
105,000 X g supernatant				
TCA soluble	125/mg	18,805/150 mg	45/mg	7,045/150 mg
TCA precipitable	2110/mg	316,815/150 mg	980/mg	142,170/150 mg

fractions. These results would indicate that to achieve adequate labelling which would insure sufficient free label available for exchange and to be sure that mating in unlabelled medium would not so dilute the radioactivity of the ribosome samples to be assayed, a greater amount of label should be used in the medium in which the homokaryon was grown prior to mating. This was feasible, since additional experiments showed that when the homokaryon was grown for 24 hr in 2 μc ^{14}C-arginine, then macerated, centrifuged and inoculated in fresh supplemented minimal medium containing 2 μc ^{14}C-arginine, additional uptake occurred.

These same assays were performed on homokaryons being prepared for mating to homokaryon grown in D_2O. The results obtained from one such experiment are compiled in Table 4. The arginine-requiring homokaryon was grown 24 hr in 2 μc ^{14}C-arginine and then in 4 μc ^{14}C-arginine for an additional 24 hr. The uptake of labelled arginine was assayed during the second 24 hr growth period and the data indicate that a considerable amount of the radioactive arginine was removed from the medium by the eighteenth hour. A slight additional amount of uptake occurred between 18 hr and 28 hr. The radioactivity of the supernatants of the washes performed prior to mating compared to the radioactivity remaining in the medium after 28 hr growth indicates that most of the radioactivity is eliminated by the washing process. This means that significant amounts of labelled amino acid would not be introduced into the unlabelled medium in which the mating was to be made. The samples of media analyzed after 12 and 24 hr of mating substantiate this since the radioactivity is quite low. The difference between the radioactivity remaining in the growth medium after 28 hr and the wash supernatants is

Table 4 Radioactivity in fractions from 250 mg ground mycelia of homokaryon, 12 hr mating, and 24 hr mating

Fraction Assayed	cpm ^{14}C-arginine X 10^{-2}							
	18 hr in 4 μc ^{14}C-arg		28 hr in 4 μc ^{14}C-arg		12 hr mating D_2O mycelium + ^{14}C-arg mycelium		24 hr mating D_2O mycelium + ^{14}C-arg mycelium	
	cpm/aliquot	Total	cpm/aliquot	Total	cpm/aliquot	Total	cpm/aliquot	Total
Culture medium	176/ml	-	133/ml	6664	14/ml	624	12/ml	477
Wash supernatant								
1st wash	-		70/ml	4906	-		-	
2nd wash	-		28/ml	1976	-		-	
3rd wash	-		12/ml	841	-		-	
15,000 X g pellet	11/mg	2691	14/mg	2945	3/mg	793	3/mg	620
ribosomes	-		161/mg ribosomal RNA	2583	25/mg ribosomal RNA	49	12/mg ribosomal RNA	23
105,000 X g supernatant								
TCA soluble	6/mg	1480	17/mg	4305	5/mg	1150	2/mg	580
TCA precipitable	26/mg	6776	33/mg	8268	13/mg	3203	10/mg	2410

approximately 2000 cpm/ml. That of the medium in which the matings were made is near this, indicating little uptake of labelled arginine during the mating.

The data indicating the radioactivity of the various fractions of the ground mycelium are of interest primarily because they provide evidence for synthesis during the mating period. Even if the radioactivity of the fractions analyzed is halved to account for the non-radioactive mycelium grown in D_2O, this would not account for the decrease in radioactivity of these fractions, expecially when the 24 hr mating mycelium is considered. The radioactivity of all the fractions assayed decreases but the most marked decrease is that of the ribosomes. The difference in the radioactivity of the ribosomes from the homokaryon going into the mating and the ribosomes derived from mycelia 12 hr after mating would appear to indicate significant synthesis of ribosomes in the non-radioactive mating medium.

It can be seen from these data that the growth of the homokaryon in greater amounts of radioactive arginine accomplished its intended purpose of increasing significantly the amount of TCA soluble radioactivity in the homokaryon. Comparison of the TCA soluble radioactivity in the homokaryon after 24 hr growth in 1 μc ^{14}C-arginine (Table 3) with that of the homokaryon after 24 hr growth in 2 μc ^{14}C-arginine + 24 hr growth in 4 μc ^{14}C-arginine (Table 4) indicates that the detectable radioactivity is increased more than 10 fold.

Analysis of Cytoplasmic Exchange During Mating

Matings were made between the fully compatible homokaryons, A41 B41 grown in D_2O-medium and A1 B1 grown in H_2O-medium containing

^{14}C-arginine. In the earliest experiments, the mating was incubated at 32°C for 24 hr and then all the flasks harvested, lyophilized, ground and the ribosomes isolated. When the ribosomes from such 24 hr matings were subjected to CsCl density gradient centrifugation, the most conspicuous result was the presence of only one visible band in the gradient. If an intense light beam was shone at an angle through the tube and the observation made in a dark room, some diffuse material was visible in a region below the obvious band. This diffuse material had the same appearance as the intense band and was assumed to be ribosomal in nature. When the absorbancy at 260 mμ and the radioactivity of fractions collected from these gradients were analyzed, results such as those shown in Figure 32 were obtained. There is a general increase in absorbancy from fraction 24 through fraction 32. This includes the density where the D_2O-labelled ribosomes banded out in previous experiments. However, there is no peak evident which could be identified as the heavy ribosomes. The radioactivity of the individual fractions also increases gradually throughout this same region of the gradient. The major peak of radioactivity appears very near the top of the gradient. The possible explanation for this is that material thought to be dissociated meniscus protein was always observed near the top of the gradient. This material remained in the tube after all fractions were collected and could be resuspended. The radioactivity in this material was always found to be greater than that of the fractions from the gradient.

The increased radioactivity of the fractions from the region where heavy ribosomes were normally located through the region where the light ribosomes were located would appear to indicate that all

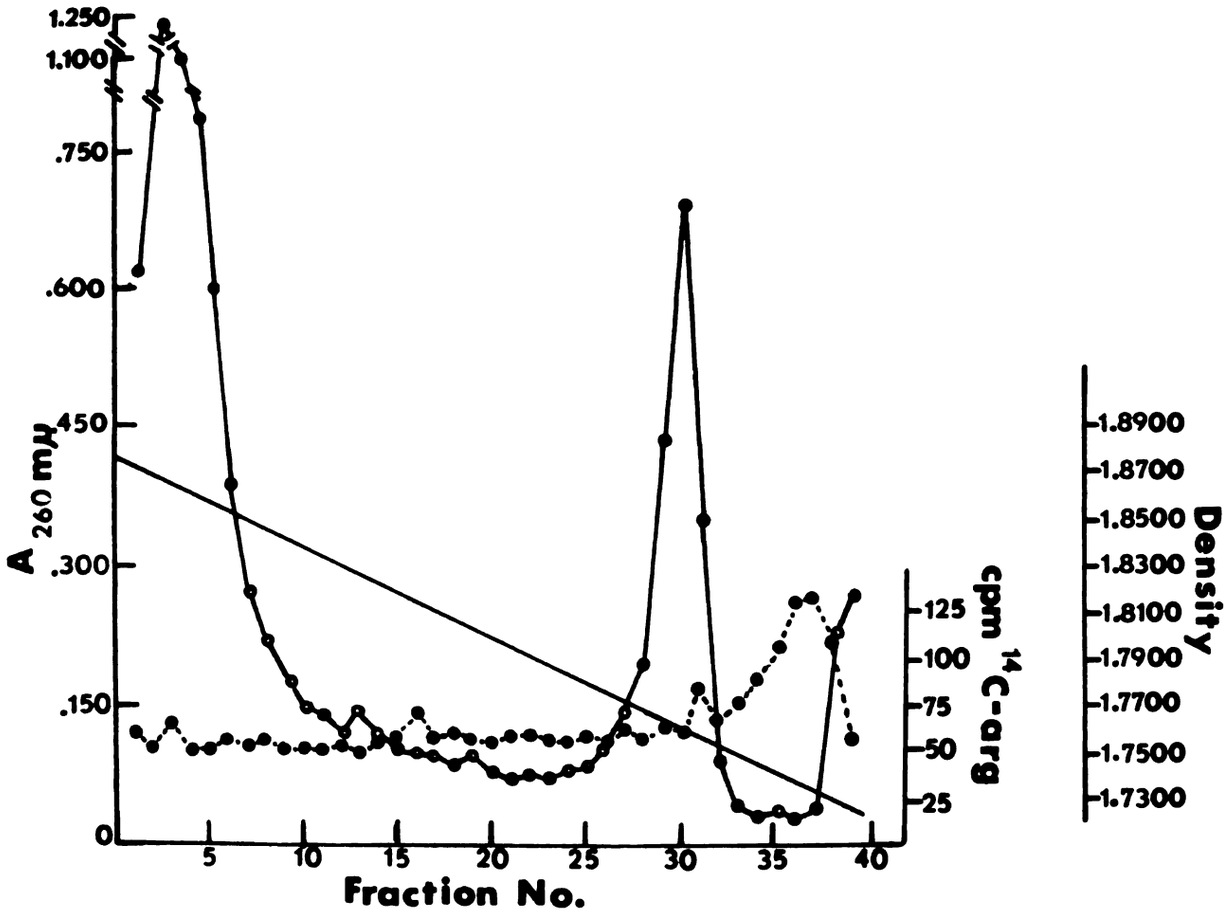
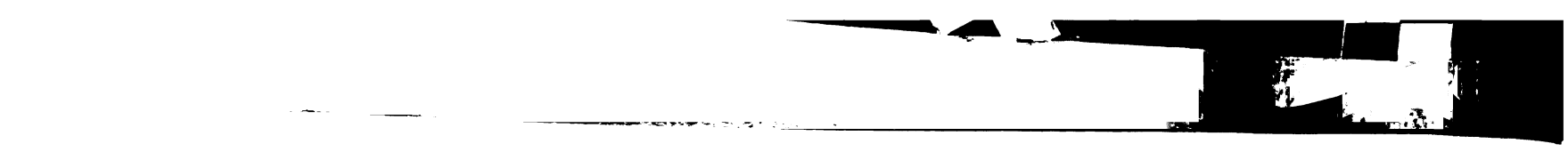


Figure 32. CsCl density-gradient profiles of ribosomes isolated from 24 hr mating of ^{14}C-arginine labelled homokaryon grown in H_2O medium and homokaryon grown in D_2O medium. (○——○, absorbancy; ●----●, radioactivity)



of the ribosomal material isolated from the mating mycelium is radioactive. This would indicate that radioactivity from the homokaryon grown in ^{14}C-arginine was available to the homokaryon grown in D_2O during the early mating interactions. Therefore, these data provide some indication for the exchange of cytoplasm during the mating process.

The failure to resolve a band of heavy ribosomes, however, was perplexing. There appeared to be two possible explanations: (1) that the growth of the homokaryon in D_2O-medium prior to making the matings did not result in sufficient density-labelling to make it possible to separate the two classes of ribosomes on the basis of density difference, or (2) that there was rapid turnover of ribosomes during the 24 hr of mating, thereby drastically reducing the number of rather uniformly deuterium-labelled heavy ribosomes. The data presented previously appeared to argue against the first possibility and to favor the second. In an attempt to resolve this question, experiments were performed in which the matings were harvested and analyzed 12 hr and 24 hr after the mating was made. In addition, part of the mycelium grown in D_2O and mycelium grown in H_2O medium containing ^{14}C-arginine was harvested prior to making the mating. The ribosomes from these mycelia would represent the 0 hr condition and differences after 12 and 24 hr of mating should therefore indicate changes which occurred during the early mating interactions.

CsCl density-gradient centrifugation of the ribosomes from homokaryons grown in H_2O-medium and D_2O-medium alone or artificially mixed gave the results already shown in Figure 24A, Figure 24B and Figure 30A respectively. It is obvious that the ribosomes from mycelium grown in D_2O are sufficiently different in density that they can be

separated into two distinct bands. This would rule out the first explanation for the disappearance of heavy ribosomes in sample from 24 hr mating mycelia. The density-gradient centrifugation of ribosomes from 12 hr and 24 hr matings supports this conclusion and provides evidence for the second alternative. The results of the centrifugation are pictured in Figure 33. In (A), ribosomes from a 12 hr mating are shown and in (B), ribosomes from a 24 hr mating are shown. As these results indicate, in both gradients one distinct band is seen near the top of the tube. Below this band a very diffuse "band" can be seen. The amount of material in the diffuse band is considerably greater in the gradient of ribosomes from the 12 hr mating than in the gradient of ribosomes from the 24 hr mating and extends further down into the gradient. Also, in the material from the 12 hr mating, a less distinct band was visible at the bottom of the diffuse "band". This lower band was not visible in the gradient of the ribosomes from the 24 hr mating. The absorbancy at 260 mμ and radioactivity of 10 drop fractions from these gradients are presented in Figure 34 and Figure 35 for ribosomes from the 12 hr mating and the 24 hr mating, respectively. The data from the gradient of ribosomes from the 12 hr mating indicate two minor absorbancy peaks, the greater of which is sharply defined at its bottom fraction but spread over 3-4 fractions in the lower density region of the gradient. The major peak, when contrasted to the minor peaks, is seen to be skewed in the opposite direction. The radioactivity increases throughout the region in which 260 mμ absorbing material appears. The The majority of the radioactivity is again found very near the top of the gradient. The data for the ribosomes from the 24 hr mating reveal differences in the absorbancy and radioactivity profiles. The most

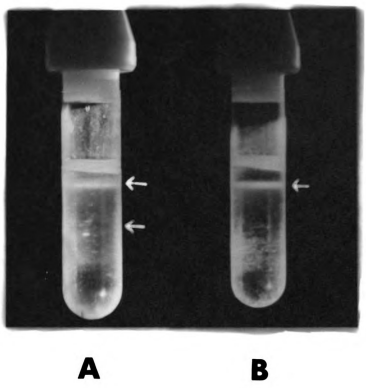
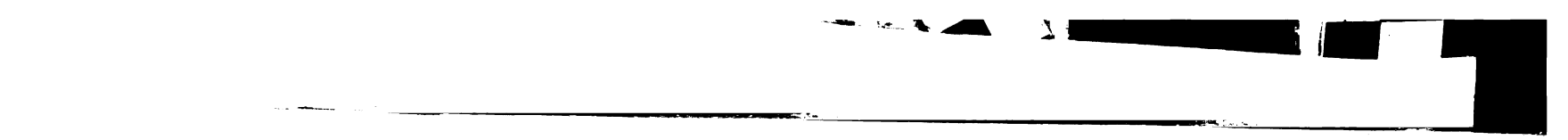


Figure 33. Results of CsCl density-gradient centrifugation of ribosomes from (A) 12 hr mating and (B) 24 hr mating between ^{14}C-arginine labelled homokaryon grown in H_2O medium and homokaryon grown in D_2O medium.



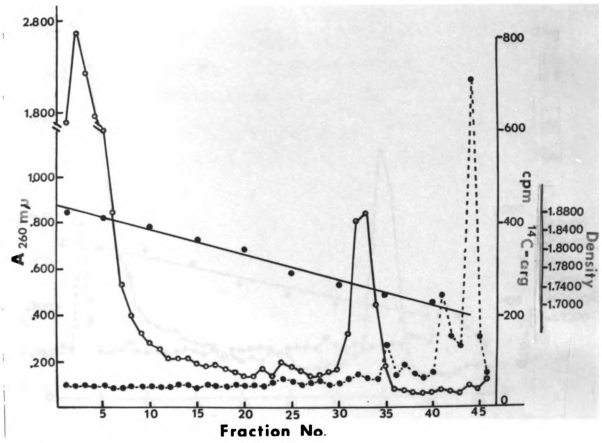


Figure 34. CsCl density-gradient profile of 12 hr mating represented in Figure 33(A). (○——○, absorbancy; ●----●, radioactivity).

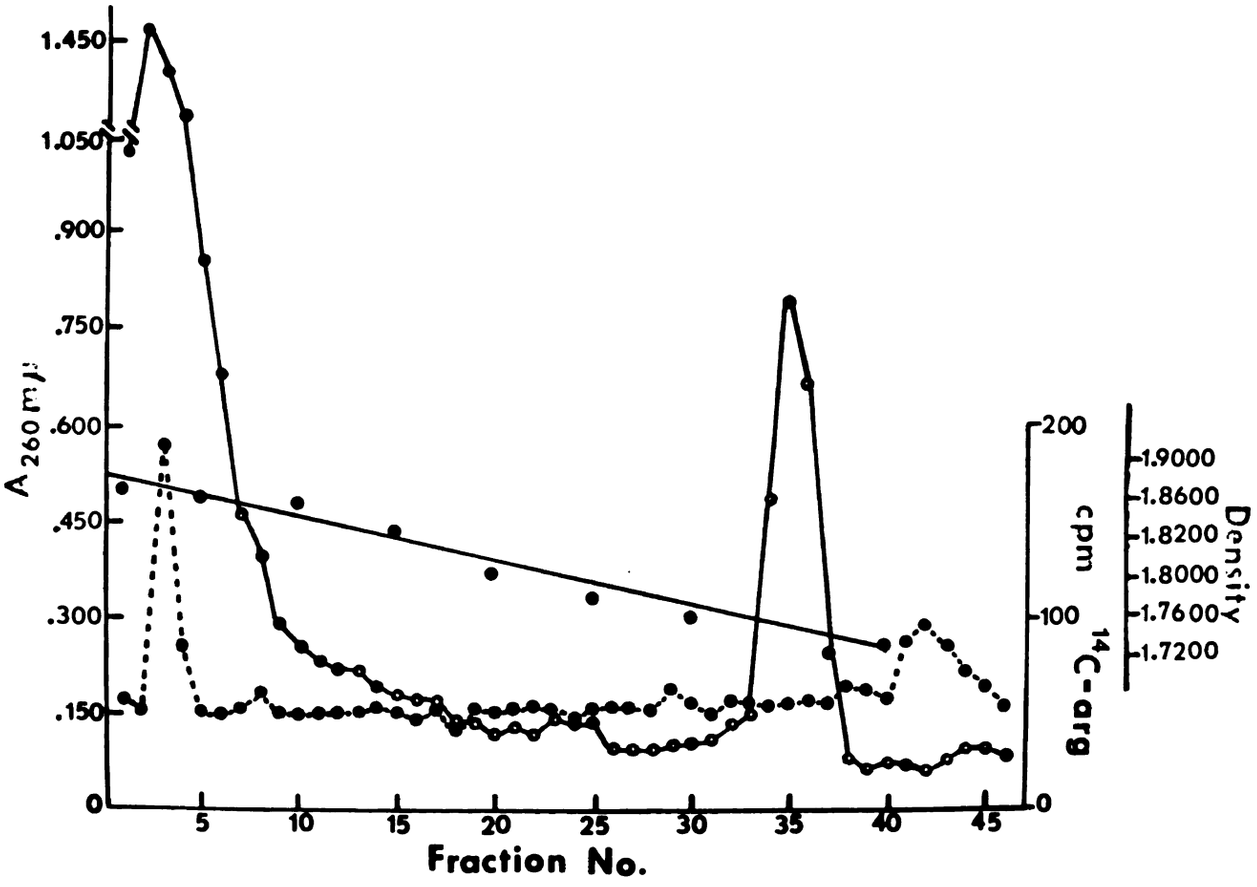


Figure 35. CsCl density-gradient profile of 24 hr mating represented in Figure 33(B). (○——○, absorbancy; ●----●, radioactivity).

noticeable difference is the disappearance of the absorbancy peak between the minor and major peaks. Instead, the major peak in this gradient is more skewed toward the bottom of the gradient. A shoulder of the major peak is present which was not present in the absorbancy profile of the gradient of ribosomes from the 12 hr mating. The radioactivity follows the pattern previously observed for ribosomes from 24 hr matings, with a generally increased radioactivity in the region of the gradient where the absorbancy peaks appear.

These data offer additional evidence for the presence of radioactivity on the density labelled ribosomes and therefore, the occurrence of cytoplasmic exchange. The difference between the material from 12 hr and 24 hr matings would appear to indicate that considerable ribosome turnover has occurred during the mating process. This would explain the near disappearance of the obvious band of heavy ribosomes and the appearance of the diffuse band of ribosomes of varying density in the material from the 12 hr mating. Further turnover would account for the decrease in the diffuse material in the additional time between the 12 hr and 24 hr mating period. Additional evidence for considerable ribosome synthesis is obtained from consideration of the total 260 mμ absorbancy in the major peaks from the gradients of ribosomes from 12 hr and 24 hr matings. As indicated in Table 5, the input of 260 mμ absorbancy material was less for the ribosomes from the 24 hr mating but the absorbancy in the peak fractions was approximately equal in the 12 hr and 24 hr mating density gradients. This indicates that ribosomal material which banded at a greater density in the gradient of ribosomes from the 12 hr mating has moved into the region of the light ribosomes. Also, these data would support those presented earlier indicating considerable

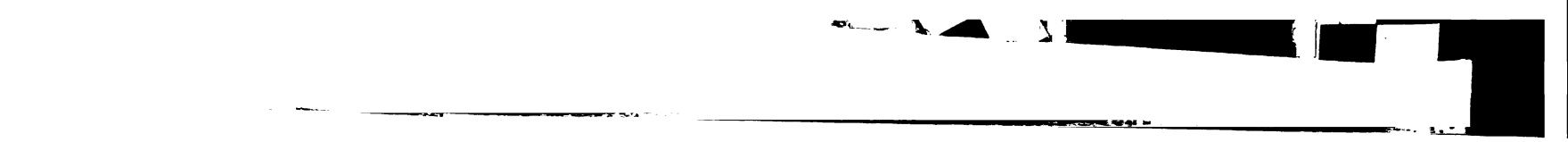


Table 5. Comparison of input and peak fraction 260 mμ absorbing material in CsCl gradients of ribosomes from 12 and 24 hr matings

Sample	$A_{260\ m\mu}$ units		24 hr / 12 hr	
	Input	Peak	Input	Peak
1st Experiment				
12 hr mating	155.92	2.907	51%	95%
24 hr mating	79.94	2.769		
2nd Experiment				
12 hr mating	158.40	1.017	82%	110%
24 hr mating	139.92	1.116		

decrease in radioactivity per mg ribosomal RNA of ribosomes due to synthesis of new, non-radioactive ribosomes during the early mating interactions.

One additional point which gives further support for the exchange of cytoplasms is the comparison of the density at which the major peaks are found. The ribosomes from Schizophyllum homokaryons and dikaryons grown in H_2O were most frequently located in the gradient at a density calculated as 1.710-1.730 g/cc. The "light" ribosomes from 12 hr mating mycelia band out at a density of approximately 1.739 g/cc, while those from 24 hr mating mycelia consistently band out at a density of approximately 1.746 g/cc. This increase in density could be accounted for by exchange of free D_2O between the mating partners and subsequent ribosome synthesis in the slightly denser combined cytoplasms. Increased exchange of cytoplasm accompanying the increased nuclear exchange demonstrated to occur would explain the increased density of the ribosomes from 24 hr mating mycelium.

DISCUSSION

The results of the experiments on the kinetics of the initial events in the mating process would indicate that the conditions under which the mating were made permit a high degree of near synchronous interactions between the mating partners early in the mating process. In all the matings studied, there was a measurable degree of nuclear exchange by twelve hours after the mating was made. That the three methods utilized for detection of nuclear exchange are valid is supported by the demonstration that the kinetics are relatively independent of the method of analysis employed. Of possibly greater significance is the demonstration that the kinetics of nuclear exchange were shown to be independent of the particular mating-type alleles and nutritional markers used.

The compatibility of the mating appears to have no effect on the earliest mating processes, i.e. hyphal anastomosis and nuclear exchange, as shown in Figure 4 and Figure 5. This apparently was assumed to be the case, since Raper states in his recent review "Following hyphal fusion and the association of nuclei from the two mates in the fusion cells, a process that is not dependent upon the incompatibility factors...." (1966b, p. 212). However, there is no direct experimental evidence for this assumption in the literature. Indeed, none of the previous studies could have demonstrated such an effect since the experimental design was such that only the later

effects of the incompatibility reaction were observed. Also, since all the earlier experiments deal with matings made on agar plates, the potential for a high degree of synchronous interaction between the mating partners is quite low. The development of the techniques used in these studies is, therefore, thought to be important in itself.

The differences among the classes of matings which appear after 24 hr of mating are consistent with the previously described properties of the incompatibility factors. It has been shown that the nuclear ratio from cell to cell in the common-A heterokaryon is very disproportionate (Raper and San Antonio, 1954). The kinetics of interaction in the common-A mating between auxotrophs probably reflect such ratios. Maceration of the developing heterokaryotic mycelium would necessarily produce a large proportion of homokaryotic fragments. The unusual fragments found to result from macerating the common-A mating would further support this idea if those fragments are assumed to be the result of limited growth of fragments in which only one cell contained more than one nucleus. In the common-B heterokaryon, it has been shown that only the tip cell is dikaryotic and dedikaryotization occurs as a result of the production of false clamps (Raper and Raper, 1964). Fragmentation of this kind of mycelium would be expected to produce predominantly homokaryotic fragments. The results obtained with the common-B and common-AB matings indicate that the restriction on nuclear migration imposed by like B factors operates in matings made under the conditions used in these studies.

The differences in the kinetics of the earliest mating interactions when the matings were compared in minimal medium and complete

medium are not completely understood. It would appear that the difference in availability of nutrients is responsible, though this relationship is only implied. One explanation is that in complete medium, the steps in dikaryosis are "repressed" by the richness of the environment, while these same reactions are "derepressed" or possibly "induced" in minimal medium. It is known that the amount of extracellular polysaccharide produced by Schizophyllum increases as the concentration of glucose in the medium is increased. Since minimal medium contains 10 times the amount of glucose in complete medium, it is possible that an increased production of polysaccharide plays some role in the altered kinetics. This role might simply be to make the hyphae "stickier", thus facilitating anastomosis. These results appear to differ from those of Wessels (1966) who presented evidence that an enzyme responsible for degradation of the cell-wall in Schizophyllum is induced by low levels of glucose. If this same enzyme is responsible for wall degradation in the formation of anastomoses, then the higher level of glucose in minimal medium apparently has no effect on its activity. Preliminary experiments in the course of the studies reported here would indicate that there is no difference in the rate of utilization of glucose when matings are made in complete or minimal medium. The difference in the two results cannot be explained at this time but it appears that further study of this phenomenon is desirable. The interactions observed are closer to being on a "per cell basis" than was possible before since the fragments being mated consisted predominantly of fewer than 5 cells. Any molecular changes found to occur as a result of the earliest mating interactions could therefore be considered to have occurred in

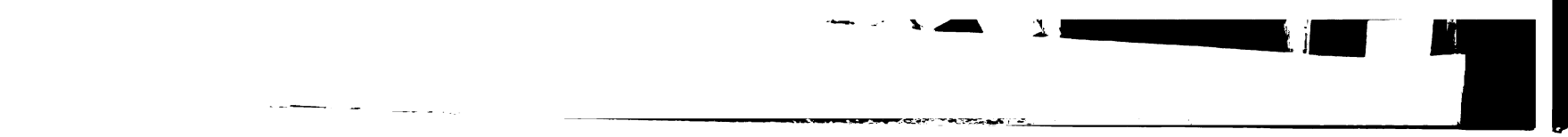
a high proportion of the cells. This would not be the case in matings made on agar media since the hyphae which interact contain many cells, only very few of which can be shown to anastomose (Sicari and Ellingboe, 1967).

It is felt that these studies on the kinetics of initial nuclear exchange represent biological evidence which supports the hypothesis that the incompatibility reaction in Schizophyllum commune is not constitutive but is induced by the presence of the two different nuclear types in the same cell. The data from these experiments were used, therefore, in designing the experiments to approach the study of the incompatibility reaction on a molecular basis.

The procedure developed for the isolation of ribosomes from Schizophyllum and Coprinus mycelia and spores made it possible to continue the studies on cytoplasmic exchange. Several aspects of the studies on ribosome isolation are interesting in themselves. The data presented indicate that intact, functional ribosomes and polysomes can be successfully isolated from lyophilized preparations of fungal material. This would appear significant since several workers have presented evidence that both the 70S and 80S ribosomes exist in vivo as highly hydrated spherical particles (for review, see Petermann, 1964). Dibble and Dintzis (1960) showed by X-ray scattering that the rabbit reticulocyte ribosome contained approximately 80% of its enclosed volume as water of hydration. Using 50S subunits of E. coli ribosomes, Hart (1962) found an internal hydration of 0.9 g H_2O per gram of nucleoprotein. The effect of drying on the structure of the ribosome in vitro has also been studied. Klug and co-workers (1961) obtained X-ray

diffraction patterns from E. coli, yeast and rat liver which showed that the ribosomal proteins maintain their structure upon dehydration, whereas the RNA molecules shrink and crumble into irregular shapes. Zubay and Wilkins (1960) obtained similar results with E. coli ribosomes and interpreted this to mean that the RNA molecules behave like DNA at high humidity and expand to adopt a regular configuration. What the studies reported here would appear to indicate is that although the structural integrity of the isolated ribosomes may be affected by dehydration, the functional integrity is not, at least when dehydration occurs in vivo. The resuspension of the lyophilized material in aqueous medium permits the isolation of functional ribosomes. Of possibly greater importance is the finding that the polysomes are maintained attached to the messenger RNA strand in the dehydrated state. That this must be the case is indicated by the polysome patterns obtained from the sucrose density gradients. The finding that the ribosomes isolated from lyophilized material still had the capacity for in vitro protein synthesis would indicate that the mRNA-polysome complex is a functional unit.

The ability to isolate polysomes from material treated as severely as was the material used in this work is contrary to most earlier reports. Whether the lyophilization which preceded the prolonged grinding plays a part in this is unknown. It is realized, however, that considerable fragmentation of the polysomes probably does occur during the grinding procedure. Therefore, the sucrose density gradient profiles may not accurately reflect the situation in vivo. Hopefully, the refinement of these techniques and the utilization of other methods of analysis such as isokinetic gradients (Noll, 1967) will permit a



detailed study of the polyribosomes in the various morphogenetic stages of Schizophyllum and Coprinus.

The presence of polysomes in ungerminated spores has been reported for only one other organism (Staples et al, 1968). The differences in ribosome profiles obtained with 0 hr, 1 hr, and 6-12 hr spores (Figures 16-20) were reflected in the capacity for in vitro protein synthesis. The significant incorporation by 0 hr spore ribosomes compared to the low activity of 1 hr germling ribosomes was reproducible. This would suggest that the protein synthesizing system is complete in the spore and needs only the proper environment to begin functioning. The failure to demonstrate a comparable number of functional polysomes in the 1 hr germling suggests that there is a very rapid turnover of the spore protein synthetic machinery and subsequent growth is needed for renewal of protein synthesis (e.g. 6 hr germlings). These differences between the 1 hr germlings and the other ages studied might be due to other factors such as the grinding procedure or some other variable in the method. Therefore, more experiments must be conducted before any conclusions on the basis for the differences can be reached. The same is true of differences which were frequently observed between ribosomes from homokaryons and dikaryons.

The study of the uptake and localization of radioactive amino acid was designed primarily to provide evidence for the feasibility of the proposed method for analysis of cytoplasmic exchange. The data indicated that radioactive amino acid was incorporated in large amounts by the fungus and that a significant portion of the amino acid remained in the TCA soluble fraction. The evidence indicating the level of protein and ribosome synthesis during the mating period is considered

important for several reasons. First, no data on protein and ribosome synthesis were available from experiments with Schizophyllum commune. Secondly, the data presented would indicate that the most obvious synthetic activity during the mating process is the formation of new ribosomes. If this is substantiated by future experiments, the rate or amount of ribosome synthesis may be implicated in the incompatibility reaction. It is possible that synthesis of large amounts of ribosomes is necessary for the events in dikaryosis to proceed normally. Finally, it is clear that significant amounts of protein are being synthesized during the period of earliest mating interactions, the first 12-24 hr of mating. It is during this period that the synthesis of a postulated incompatibility protein should occur. Therefore, taking these data together with the data from the kinetics studies, any experiments designed to obtain information on the molecular basis of incompatibility should be conducted in the first 24 hr of mating.

The appearance of radioactivity throughout the region of the gradient in which the heavy and light ribosomes were localized provides some indication that the radioactivity in the TCA soluble fraction of the light homokaryon was transferred to the other homokaryon during mating. Also, the change in the density at which the "light" ribosomes band in the gradient indicates that D_2O was exchanged and utilized by the entire "mating mycelium" for ribosome synthesis. However, these experiments can only be considered as a preliminary investigation providing very limited evidence for exchange of cytoplasm in the earliest mating interactions. Before substantial evidence can be obtained, many of the anomalous results from CsCl density-gradients must be explained. These include the appearance of radioactivity in very dense regions of

the gradient and the displacement of radioactivity peaks from absorbancy peaks. The most important variable to be eliminated is the observation that CsCl density-gradient centrifugation of artificially mixed light radioactive and heavy non-radioactive ribosomes can indicate radioactivity in the region between the light and heavy ribosome absorbancy peaks. An example of this is shown in Figure 36. In this experiment, the major peak of radioactivity is displaced into the denser region of the gradient between the absorbancy peaks representing the heavy and light ribosomes. The data from this experiment are incomplete, as no analysis of the density of the fractions was obtained. Also, the radioactive homokaryon ribosomes were not centrifuged alone to see where the radioactivity appeared. Results such as these, however, indicate that much additional work must be done to eliminate such variables before these methods can be used for further studies. A primary concern is insuring the stability of the ribosomes to eliminate breakdown into fractions which band out at very dense regions of the gradient and at the meniscus. This might be accomplished by fixing the ribosomes in formaldehyde prior to CsCl density-gradient centrifugation (Perry and Kelley, 1966, 1968) and/or centrifugation of the ribosomes through sucrose gradients prior to CsCl density-gradient centrifugation to eliminate non-ribosomal material. It is also essential to use more pure, optical-grade CsCl to reduce non-ribosomal material which absorbs at 260 mμ and is thought to be responsible for the very high absorbancy in the lower half of the gradient.

Possibly the most significant data obtained from the CsCl density gradient analysis of ribosomes derived from matings between deuterium-labelled and ^{14}C-arginine labelled homokaryons is the evidence for

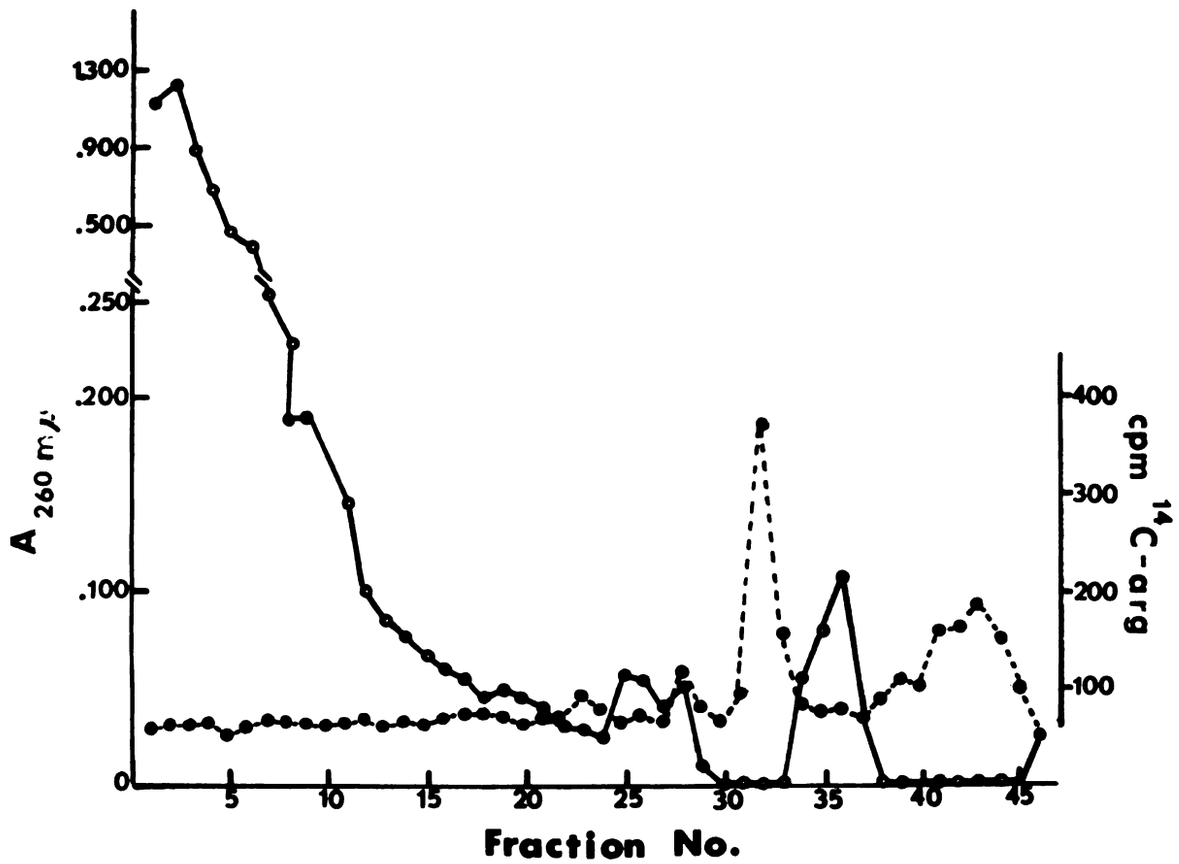
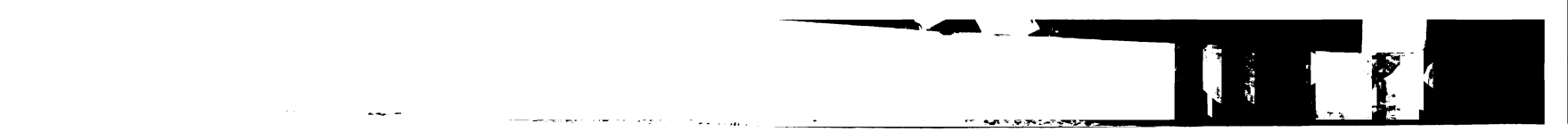


Figure 36. CsCl density-gradient profile of an artificial mixture of ribosomes from ^{14}C-arginine labelled homokaryon grown in H_2O medium and homokaryon grown in D_2O medium. (O——O, absorbancy; ●----●, radioactivity).

rapid replacement of the heavy ribosomes with ribosomes of hybrid density. Eventually, this results in the virtual disappearance of the heavy ribosomes. Kaempfer and co-workers (1968) have shown that during growth, the ribosomes of E. coli regularly dissociate and reassociate during growth. Their evidence is based on the disappearance of deuterium-labelled ribosomes with an accompanying appearance of ribosomes of hybrid density. The basis for disappearance of heavy ribosomes is that the heavy dissociated subunits reassociated with light subunits dissociated from ribosomes formed after transfer from heavy medium to light medium. This is very similar to the situation observed in the experiments reported here. The evidence was presented for rapid ribosome synthesis during the mating period, and cyclic dissociation-reassociation of heavy and light ribosomes would result in decrease of the heavy ribosomes and increase in the ribosomes of hybrid density. Continued synthesis of ribosomes in H_2O-medium would make the concentration of heavy ribosomes limiting and eventually result in near disappearance of heavy ribosomes. Some would be present for extended periods, however, since D_2O turnover with H_2O is slow if the D_2O is incorporated into protein. This explains the observation of very small concentrations of heavy ribosomes after 24 hr of mating.

The existing evidence indicates that the ribosomal subunits are conserved intact during dissociation and reassociation (Kaempfer, Meselson, and Raskas, 1968; Kaempfer, 1968; Meselson et al, 1964). If this is the case, it would be expected that the four classes of ribosomes consisting of all heavy subunits, heavy large subunit + light small subunit, heavy small subunit + light large subunit, and



all light subunits could be resolved in the gradient. No data to support this appear in the literature. It is possible that subunit reassociation is the primary mechanism in the formation of hybrid ribosomes but that some dissociation of protein and RNA does occur. This type of dissociation has been observed (Perry and Kelley, 1968; Samarina et al, 1967) and could account for the observed gradual increase in radioactivity throughout the region in which the heavy, hybrid and light ribosomes appear. Kaempfer (1968) showed that during protein synthesis the ribosomes undergo considerable subunit exchange and proposes that ribosome subunit exchange is a part of the mechanism of protein synthesis. Kaempfer's experiments involved analysis of in vitro protein synthesis but it appears that the explanation for subunit exchange can be extended to an in vivo system in the experiments reported here since data were presented which indicate that considerable protein synthesis does occur during the 24 hr mating period. An experiment which might provide more evidence for subunit exchange would be to label the mycelium grown in D_2O with ^{32}P just prior to mating. Analysis of the ribosomes derived from the "mating mycelium" at various times after mating should reveal a decline in ^{32}P-label in the heavy ribosomes and appearance of increased amounts of label in hybrid ribosomes. If label appears in the light ribosomes, this would be additional evidence for cytoplasmic exchange. If the mating was labelled with ^{3}H-uracil to put the label in RNA which is not stripped off the ribosome by CsCl, the newly synthesized ribosomes should incorporate the label, thus providing better evidence for the degree of ribosome synthesis during the mating period.

It is felt that these studies on the earliest mating interactions have served to provide further evidence for the induced nature of the incompatibility reaction, to develop valuable techniques for study of the protein synthesis system in Schizophyllum and Coprinus, and to approach the study of incompatibility on the molecular level from a new direction. It is realized that these last experiments provide only very preliminary evidence and have resulted in more questions than answers. Perhaps that is their greatest contribution.

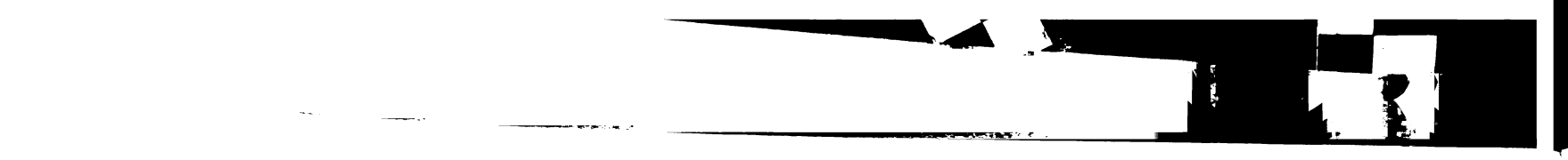
SUMMARY

The kinetics of nuclear exchange were investigated in all 4 classes of matings in Schizophyllum commune. These kinetics are different in the compatible mating vs. the 3 non-compatible matings and in the common-A mating vs. the common-B and common-AB matings. No difference in the common-B and the common-AB matings was observed. In all 4 classes of matings, a significant percentage of fragments had interacted and exchanged nuclei 12-24 hr after mating began. There was no detectable effect of the particular compatibility alleles or nutritional markers used on the kinetics of nuclear exchange. Hyphal anastomosis and nuclear exchange occurred more rapidly and synchronously in minimal medium than in complete medium.

It was shown that intact ribosomes and polysomes could be isolated from lyophilized mycelia and spores of Schizophyllum and lyophilized mycelia of Coprinus. The monosomes which predominate in these preparations have an approximate sedimentation value of 80S. Polysomes of up to 4 ribosomes in size which are stable in low Mg^{+2} and sensitive to ribonuclease treatment are also present. The ribosomes isolated from all sources are functional in in vitro protein synthesis without added messenger RNA.

Analysis of possible cytoplasmic exchange during mating indicates that such exchange does occur by twelve hours after mating was begun. When matings were made between a D_2O-labelled homokaryon and a

^{14}C-arginine labelled homokaryon, radioactivity was present on the heavy ribosomes after 12 and 24 hr of mating. Further evidence for cytoplasmic exchange was provided by the data indicating that the "light" ribosomes increase in density as the mating time increases. The appearance after 12 hr of mating of ribosomes of hybrid density which are radioactive and their disappearance in another 12 hr of mating indicates that considerable ribosome synthesis and turnover accompanies the early mating interactions.



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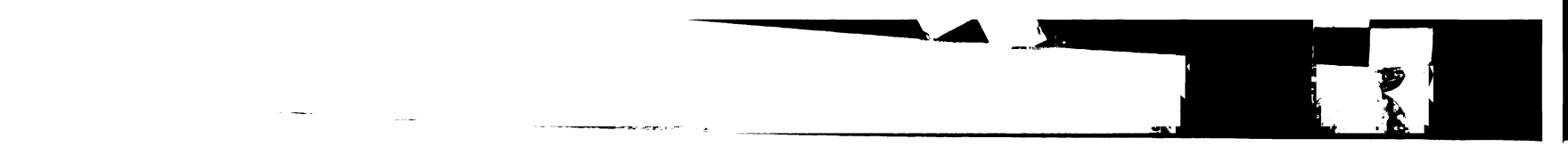

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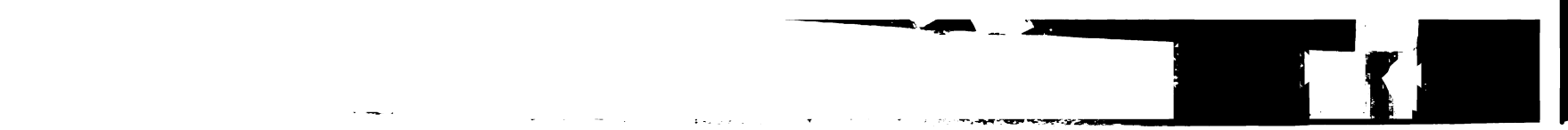

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