Parasexual Cycle in Dermatophytes

The work submitted presents for the first time proof of the existence of parasexual cycle in dermatophytes, fungi parasitic for man and animals. Our findings pave the way for solving a series of practically important problems by genetic analysis (genetic control of virulence, morphogenesis, etc.). The parasexual cycle includes: origin of heterokaryons; formation of heterozygous diploids; segregation and recombination at mitosis. Quoted hereinafter are brief proofs relating to all three phases of the cycle.

For the preparation of mutants we used the wild strain MG-155 (*a, cre*) of the dermatophyte *Microsporum gypseum* (Bodin) GUIART et GRIGORAKI 1928 (perfect st. *Nannizzia incurvata* Stockdale 1961). This strain is prototrophic and abundantly produces microconidia[1]. By means of UV-radiation we prepared 69 auxotrophs with colour markers[2,3].

With the help of a micromanipulator a direct proof of heterokaryosis was realized by isolating hyphal tips. Nutritional complementation on minimal medium and morphological complementation on complete medium (CM) was used to indicate indirectly the heterokaryotic constitution. Growing sectors originated more quickly during growth of the heterokaryons on minimal medium (MM). Data given testify to their diploid constitution.

The phenotype. Heterokaryons on MM are always noted for reduced growth rate and heterogenous morphology. Growth and morphology of diploids are identical both on MM and CM with the wild strain MG-155: cream and prototrophic colony with abundant microconidia. Heterokaryons easily dissociate into haploid components; the diploids, however, are constant.

Conidial size[4]. From each strain 100 microconidia were measured (Table). The length of the conidia is in all diploids significantly greater than in the haploid strain. The change in length, however, was not sufficient to allow the possibility of using it as a factor to determine ploidy in mitotic segregants. The width of the conidia remained unchanged.

Nuclear size. The nuclei were coloured by HCl-Giemsa[5]. The nuclear diameter was measured in twenty microconidia from each strain. As to diploids, the volume of the nucleus (calculated as volume of sphere) was 1.4–1.6 times greater than in the haploid strain (Table).

Mitotic recombinants. Heterokaryotic constitution is not transferred through microconidia. Only coloured colonies of both the auxotrophic components were found growing in conidial spreads. Most colonies with complementary phenotypes were formed in spreads from diploids. The frequency of segregants varies in accordance with the genotypes of diploids. From each of the 12 diploids under study, we obtained mitotic recombinants. On the examples in conidial spreads from diploids *yel bio/vio ino* (prepared from mutants 6/39 and 7/4) we isolated classes of segregants of the following phenotypes: 1. *vio*$^+$ *yel*$^+$ *bio*$^+$ *ino*$^+$; *vio*$^+$ *yel bio ino*$^+$; *vio yel*$^+$ *bio*$^+$ *ino*; 2. *vio*$^+$ *yel*$^+$ *bio ino*$^+$; *vio*$^+$ *yel bio*$^+$ *ino*$^+$; *vio*$^+$ *yel bio ino*; *vio*$^+$ *yel bio ino*$^+$; 3. the colonies of 2 new morphological types (marked m_1, m_2): m_1 *ino bio*; m_2 *ino bio*; m_2 *ino*$^+$ *bio*.

The occurrence of mitotic recombinants yields a convincing proof of diploid constitution. In *Microsporum gypseum*, heterokaryons and diploids very often originate under standard cultivation conditions. We surmise that this may be the case even with other species of these parasitic fungi.

Zusammenfassung. Die diploiden Stämme und die von ihnen gewonnenen mitotischen Rekombinanten beim Dermatophyten *Microsporum gypseum* werden beschrieben.

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Size of microconidia and nuclei in haploid and diploid strains

Genotype	Ploidity	Length of microconidia (μm)	Diameter of nucleus (μm)	Volume of nucleus (μm^3)
ino cre	*n*	3.7	1.1	0.73
bio och/ino yel	2 *n*	5.7	1.3	1.19
bio vio/ino bre	2 *n*	5.2	1.3	1.04
bio lye/ino bre	2 *n*	4.6	1.3	1.06
bio yel/ino cin	2 *n*	5.3	1.2	1.01
bio vio/ino lye	2 *n*	4.1	1.3	1.04
bio lye/ino cin	2 *n*	4.2	1.3	1.09

n, haploid; 2 *n*, diploid

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Stability of Tritiated Thymidine During Prolonged Labelling of Human Blood Leukocyte Cultures

Since 1960 tritiated thymidine has been used extensively for studying the replication of chromosomes[1]. The most widely used method of labelling chromosomes is a 20 min incubation with tritiated thymidine. Recently a prolonged labelling method in which cells are incubated for 50 to 72 h in medium with radioactive thymidine has been introduced[2–6]. The implication of studies using the prolonged incubation method is that little or no degradation of labelled thymidine occurred. However, to our knowledge, the only published data on stability of tritiated thymidine in cultures showed a rapid degradation of the label to tritiated thymine[7]. This study used suspensions of either dog bone marrow cells, dog thoracic duct lymphocytes or human leukemic blood in autologous se-

rum. It therefore seemed appropriate to reinvestigate the stability of radioactive thymidine under the usual conditions of normal human leukocyte cultures.

20 ml of heparinized venous blood was collected and the leukocytes were cultured by the method of MOORHEAD et al.[8]. Cells were diluted to a final concentration 1.5×10^3 mm^3 in Minimum Essential Medium (Grand Island Biological Co.) to which 15% fetal calf serum, phytohemagglutinin-M 2% final concentration (Difco No. 485336) and antibiotics (50 U penicillin and 50 µg streptomycin per ml) were added. Tritiated thymidine (2.0 Ci/m-mole, New England Nuclear) was added to a final concentration of 0.1 µCi/ml. Duplicate aliquots of the cell suspension were harvested at 0, 5, 28, 51 and 72 h after onset of incubation. The cells were separated from the medium by centrifuging at 2000 rpm at 4 °C. Radioautographs of cells prepared after 72 h of growth in medium with tritiated thymidine showed that nearly all cells had incorporated between 100–200 grains per nucleus. The radioactivity in 0.1 ml of medium from each sample was determined by scintillation counting in 15 ml of Bray's dioxane solution[9]. 5 mg of unlabelled thymidine and thymine were added to each ml of medium as carrier and the sample was vigorously boiled for 30 min to deproteinize it. The solution was clarified by centrifugation and 50 µl of the supernatant was dried as a 1 cm spot on WHATMAN No. 1 paper. Ascending chromatography was carried out using a solvent system described by FINK et al.[10], which is the upper phase from a mixture of ethyl acetate, water and formic acid (60:35:5). This system was selected since it provides the best separation of thymidine from its major degradation product thymine. The chromatograms were dried and examined under UV-light to locate the thymidine and thymine spots. Each spot was cut from the chromatograms and placed in a toluene based scintillation fluid. Internal standards were used to measure quenching by medium and paper.

The Table shows that the total tritium content of the medium is essentially unchanged during 72 h of incubation. Moreover, nearly all the radioactivity in the medium is tritiated thymidine throughout the 72 h of incubation. These results indicate that in human leukocyte cultures the prolonged labelling method is valid since there is little depletion or degradation of label. The difference between the present study and the previous results of RUBINI[7] are probably explained by marked differences in the methods of culture. RUBINI used high concentrations of cells in relatively small volume of medium and many of his preparations were rich in granulocytes which contain high thymidine phosphorylase activity[7]. On the other hand, our studies were carried out under the usual conditions used for leukocyte cultures and chromosome preparations.

Zusammenfassung. Der Stoffwechsel des ^{3}H-Thymidins in Blut-Leukozyten wurde während langfristiger Inkubation studiert. Die Bedingungen waren dieselben, die gewöhnlich for Chromosomenpräparate angewendet werden. So gelang es, menschliche Leukocyten während 72 Stunden mit H^3-markiertem Thymidin zu inkubieren, ohne dass das markierte Thymidin durch die Leukozyt-Thymidin-Phosphorylase zerstört wurde.

Determination of tritiated thymidine in medium from lymphocyte cultures at various times

Time of incubation (h)	Total cpm in medium[a]	Total cpm in thymidine[b]	Total cpm in thymine[b]	Total cpm in thymidine + thymine
0 h (no cells)	52,422	47,622	1,404	49,026
0 h (with cells)	66,353 ± 2,945	54,376 ± 216	1,438 ± 180	55,814 ± 198
5 h (with cells)	75,932 ± 2,076	71,964 ± 5,196	1,022 ± 168	72,986 ± 2,682
28 h (with cells)	88,849 ± 9,648	52,416 ± 10,260	3,628 ± 936	56,194 ± 5,598
51 h (with cells)	69,903 ± 5,134	58,916 ± 2,772	2,052 ± 180	60,968 ± 1,476
72 h (with cells)	74,772 ± 2,686	53,028 ± 5,292	1,836 ± 288	54,864 ± 2,790

[a] The average of 2 duplicate determinations and their standard deviations is shown in the first 3 columns of the table. Thymidine and thymine were separated by ascending chromatography as described in the text. The Rf values for thymidine and thymine were 0.275 and 0.44, respectively. [b] All data were corrected for quenching as determined by the use of internal standards. The efficiency of counting for tritium was approximately 60%.

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