Sex hormones in N. crassa

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Abstract
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This morphological mutants and systems for studying differentiation is available in Fungal Genetics Reports:
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Peduzzi, R. and G. Turion. Conidiation antigen and malate dehydrogenase isoenzyme activities.

We have recently detected on arc of precipitation produced by an antigenic compound found in the normally conidiating wild type strain Lindgren A of N. crassa but lacking in the morphologic 1 oconidiol mutant amyc. (isol, #422). The arc was found to reappear simultaneously on amyc acid and succinate medium (Peduzzi and Turian, in press). This antigenic compound is also present in the protein extract of two other normally conidiating strains (isol, #15300; isol, #21-Changins, Switzerland). (Gindrat et al., Mycopathol. Mycol. Appl., in press).

It was therefore interesting to attempt to characterize biochemically this conidial antigen in an effort to understand its eventual physiological role. For that the extract for immunization was prepared by 3 successive freeze (liquid nitrogen)-thawing (30°C) operations followed by lyophilization of the mycelium which was then ground and the resulting Powder extracted with phosphate buffer 0.06 M, pH 7.2, and spun at 10,000 x g in the cold. The method of detection of enzymatic activities on the immunoelectrophoretic patterns according to Uriel (1964 immunoelectrophoretic analysis. p. 30. In Grabar and Burtin, Immunoelectrophoretic analysis. Elsevier, New York) has been used to establish the precipitation arcs as enzyme-antibody complexes.

Of the many dehydrogenases which have also been tested, only the malate dehydrogenase (MDH) has been found to be active at the level of the specific arc of precipitation present in the wild type immunoelectrophoretic analysis (E.E.A.) patterns. However, in addition to this low cathodic mobility MDH-positive arc (MDH2), two other arcs also show MDH activity in such patterns; these correspond to the MDH isozymes (MDH1 and MDH2) already recognized with the technique of acrylamide separation in wild type N. crassa extracts (Kittel et al. 1967 Arch. Biochem. Biophys. 121: 224; Strickland and Shields 1967 Neurupora Newsl. 12: 15). Using the same technique, Loycock et al. (1963 Neurupora Newsl. 4: 20) have detected a weak addition 1 isozymic bond (MDH4), along with the technique of which has found using starch gel electrophoresis. On immunochemical grounds, however, it is known that two isozymes can react as a single antigen (Pfeiderer et al. 1966 Biochem. Z 346: 269).

By contrast the I.E.A. pattern of the protein extract of the amyc mutant developed with the homologous antiserum to normally oconidiol amyc on sucrose medium shows only one positive MDH arc, with low anodic mobility, corresponding to MDH1 also seen in the wild type pattern. However, when oamyc is induced to conidiote on acetate + succinate medium, its I.E. A. pattern (developed with antiserum of the wild type, containing the conidia specific antibody) shows two MDH positive arcs, not only MDH1 but also a well defined MDH2, as recognized by its cathodic mobility and characteristic location.

In conclusion, phenotypic reversion of oamyc to conidiolation is accompanied by the appearance of a new enzymatically active protein arc. This protein (MDH2) is induced by acetate simultaneously with the induction of the glyoxylate cycle and the associated processes of gluconeogenesis (Witt et al. 1966 Biochem. Biophys. Acta 128: 63). These phenomena of particular significance for an understanding of the metabolic orientation required for conidiolation. — Laboratoire de General Microbiology, University of Geneva, Geneva, Switzerland.


Sex hormones (i.e., sex- and fertility-inducing substances) have been isolated from cultures of crosses between Em A and Em a as well as from the homokaryons of Em A and Em a of N. crassa. For the extraction of sex- and fertility-inducing substances from on established background, 15 ml of sterilized liquid cross medium were poured into a single Petri dish (size 10 x 15 mm.) in which 5 sheets of sterilized filter paper (1, 9 cm.) were placed for the support of mycelial growth. Inocula of both Em A and Em a were made simultaneously on the filter paper close to one another and the culture was incubated at a temperature of 23-26°C for three weeks, by which time free ascospores could be detected. A similar procedure was used for single strain cultures (either Em A or Em a), except for the fact that a small medium was used.

Extraction was made after three weeks of incubation of the cultures. The filter paper (50 x 5 of them) with the adhering mycelia were soaked in 3000 ml. of sterilized distilled water for about two hours under occasional stirring with a glass rod without disturbing the integrity of the filter paper and the adhering mycelia. Subsequently, the water was decanted and the filter papers with the adhering mycelia were soaked again in an additional 3000 ml of sterile distilled water. In total, 6000 ml of water-extract was obtained in this manner. The water-extract was then filtered through a filtering apparatus composed of 8 layers of cheese cloth together with a thick glass wool pad and, finally, 3 layers of filter paper #1.

Five grams of activated charcoal (Norit-A) were added to 500 ml of the water extract. The mixture was agitated for a few minutes after which it was kept at room temperature for about 3 hours. Subsequently, as much water as possible was decanted without disturbing the charcoal sediment. The remaining water together with the charcoal was then filtered through filter paper #1 and the charcoal so collected was dried at room temperature. 300 ml of chloroform (reagent grade) were added to the dried charcoal and agitated vigorously for a few minutes, after which the mixture was kept at room temperature for about 3 hours with occasional and mild agitation, after which the chloroform mixture was filtered twice through 5 layers of filter paper #1. This extraction procedure was repeated once more and, finally, about 500 ml of chloroform extract was obtained in this manner. The extract was then evaporated to dryness under vacuum suction. The residue, consisting of a
thin film of an oily substance was redissolved in 15 ml of chloroform, collected in a specimen vial and kept under refrigeration.

The extracts were tested for their biological activity by dispensing 0.1 ml (2 pgm, dry weight) of extract on a small triangular piece of filter paper (71) placed in the center of a test plate. The test plates contained 20 ml of Westergaard's crossing medium and were simultaneously inoculated with Emerson A and Emerson a. Each experiment was repeated at least 5 times. The crosses were allowed to grow at room temperature (23-26°C) for three weeks. Observations were made at 7-day intervals. In addition, the extracts were tested on single strain cultures of either Em A or Em a grown on solidified minimal medium. The control consisted of the addition of a small triangle of filter paper onto which 0.1 ml of chloroform was pipetted.

For biochemical tests, the extracts were purified. The method followed was basically that of Folch et al. (1957) for the purification of lipids. Re-distilled water (0.2 ml by volume) was added to the final extract with thorough mixing and the mixture was allowed to separate into two layers for 15-30 minutes, after which the upper layer (water) was siphoned off carefully and discarded. The process was repeated once more, after which the solvent layer was stored under refrigeration.

The extracts were found to possess the following biological properties: (1) The extracts, when applied to cultures of single strains of Em A or Em a, induced a ten-fold increase in the number of protoperithecia and protoperithecia-like bodies developed by strain Em A; no increase was noted when Em a was used as the tester strain. (2) The extracts, when applied to crosses between sterile and wild type strains, improved the fertility (as measured by the number of perithecia with spore content) of some sterile strains of mating type A but not of a sterile strain of mating type a, when compared to untreated controls. (3) The extracts, when applied locally to a fertile cross (Em A x Em a), gave a five- to ten-fold increase in the number of mature perithecia developed by the cross. In addition, the extract caused a chemotactic response in terms of localized development of perithecia on the treated filter paper. (4) In an isolated case, the extract from a cross Em A x Em a was found to induce 'selfing' of an Em A strain, thereby giving rise to A, a, bisexual (self-sterile) and sterile progeny. No perithecia were observed in control cultures treated with chloroform only. In all cases the plating test for bacterial and fungal contaminants of the extract remained negative.

Biochemical investigations using thin-layer chromatography (3:1 chloroform-benzene) indicated that two substances in the cross extract (Em A x Em a) moved with the solvent front. Chromatography of each of the single strain extracts (Em A or Em a) yielded only one biologically active spot moving with the solvent front. The chromatography spots yielded a lipid-positive color test with spray reagents. Ultraviolet spectrometry, infrared spectrometry, nuclear magnetic resonance, mass spectra determinations and microanalysis of the extracts of single strains (Em A and Em a) characterized the sex-inducing substances to be open-chained, unsaturated, and possibly branched hydrocarbons with a molecular weight of 354-372 (mating type a) and 344-357 (mating type A). All tests confirmed a highly satisfactory degree of purification of these substances by the methods employed.

Recently we have developed techniques of DNA:DNA and DNA:RNA hybridization and of chromatin isolation permitting studies on a molecular basis of differentiation in Neurospora, in collaboration with D. E. Kohne of the Department of Terrestrial Magnetism, Carnegie Institution, Washington, D.C. and D. P. Bloch of the Institute of Cell Research, University of Texas, Austin, Texas. These techniques have made the following studies possible:

(1) Studier on repeated DNA sequence in N. crassa. While most eucaryotic organisms contain large numbers of repeated DNA sequences, N. crassa has very few (Dutta and Kohne 1969 Proc. XI Intern. Botany Congr. 1969:50), if any, of such repeated sequences. Approximately 10% of the whole cell DNA is found to be repeated. This is believed to be mostly mitochondrial DNA. This will be an extremely useful property in the interpretation of the nucleic acid hybridization data. Furthermore, it has been possible to study the entire kinetics of DNA reassociation. This knowledge enables an accurate measurement, within 1% error, of the identity of nucleotide sequence of DNA from different cell types. Comparisons of half Cot values (Cot = OD at 260 m&/2)x hours of incubation / 1/2 Cot = Cot value for 50% hybridization) of E. coli (standard) DNA with N. crassa DNA enable us to conclude that the "information content" of N. crassa nuclear DNA is close to 2 x 10^{10} daltons. This indicates that N. crassa nuclear DNA will take 15 hours, in comparison with 750 hours for DNA of the cow, in order to get 95% DNA:DNA reassociation at a concentration of 5 mg DNA/ml in 0.18 M sodium ion. Bored on the some technique, we have found that the information content of Neurospora mitochondrial DNA is 7 x 10^{12} and that there are only 30 copies of DNA repeats per cell.

(2) Studies on differential gene expression by DNA:RNA hybridizations. The earlier studies made with higher organisms on this problem are based on DNA-agar and membrane filter techniques measuring only the expression of repeated sequencer of DNA. Using these techniques, we have not been able to obtain more than 30% DNA:DNA hybridization compared with the 99% easily obtained by the hydroxyapatite technique (Britten and Kohne ibid.) between the identical DNA. It should be possible to isolate RNA cistrons from different cell types of Neurospora by this technique, using the procedure of Kohne (1968 Biophys. J. 8: 1104).

(4) Studies on chromatin isolated from differentiated cells of N. crassa. Several workers have established the usefulness of the study of the chemistry of chromatin for understanding the molecular basis of morphogenesis in higher organisms. Our studies regarding the chemical composition of chromatin and basic proteins (Dwivedi, Dutta and Bloch 1969 J. Cell Biol. 43:51) in-