Nucleic acid interactions and chromatins isolated from differentiated cells

S. K. Dutta
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Abstract
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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 µgm, dry weight) of extract on a small triangular piece of filter paper (8) 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 sterile 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 one 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.

Dutta, S. K. Studier on nucleic acid interactions and chromatin isolated from differentiated cells. 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) Studies 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 sequences of DNA from different cell types. Comparisons of half Cot values (Cot = OD at 260 µg/ml x hours of incubation / 1/2 Cot = Cot value for 50% hybridization: Britten and Kohne 1968 Science 161:529) of 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 99% DNA:DNA reassociation at a concentration of 5 mg DNA/ml in 0.18 M sodium ion. Bored on the same technique, we have found that the information content of Neurospora mitochondrial DNA is 7 x 10^9 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 sequences 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's. 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. B. 11:104).

(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-
dicate that probably some different kind of basic proteins (other than any known histones) are involved in such lower eucaryotic organisms. We have shown (Dutta and Crockett 1968 The Nucleus, p. 65, Calcutta Univ. Seminar Vol.) that there are some differences in chemical constituents of DNA and RNA in chromatin isolated from mycelial and conidial cells.

All of these studies indicate very strongly the value of working with Neurospora cell types and morphological mutants to gain useful knowledge regarding the molecular basis of differentiation. Part of these studies are already published, and parts are in the process of publication elsewhere. This research has been supported by a NSF grant GY3894.  – – – Department of Botany, Howard University, Washington, D.C. 20001.