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Abstract

Nucleic acid interactions and chromatins isolated from differentiated cells

Dutta, S. K. Studier on nucleic acid interactions and chromatinr 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 studier 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 enabler an accurate measurement, within 1% error, of the identity of nucleotide sequencer of DNA from different cell types. Comparisons of half Cot values (Cot = OD at 260 mμ/2)x hours of incubation: 1/2 Cot = Cot value for 50% hybridization: Britten and Kohne 1968 Science 161:529) 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^{7} and that there are only 30 copier of DNA repeats per cell.

(2) Studies on differential gene expression by DNA:RNA hybridizations. The earlier studier 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 hove not been able to obtain more than 30% DNA:DNA hybridization compared with the 98% easily obtained by the hydroxyapatite technique (Britten and Kohne ibid.) between the identical DNAs. It should be possible to isolate RNA cistrons from different cell types of Neurospora by this technique, using the procedure of Kohne (1968 Biophyr. J. 8: 1104).

(4) Studies on chromatinr isolated from differentiated cells of N. crassa. Several workers have established the usefulness of the study of the chemistry of chromatins for understanding the molecular basis of morphogenesis in higher organisms. Our studier regarding the chemical composition of chromatinr 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) ore 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 chromatins 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 there studies are already published, and ports 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.