

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 In-

stitution, 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 ($Cot = (OD \text{ at } 260 \text{ m}\mu/2) \times \text{hours of incubation}$; $1/2 \text{ Cot} = \text{Cot value for 50\% hybridization}$; 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 2×10^{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. Based on the same technique, we have found that the information content of *Neurospora* mitochondrial DNA is 7×10^7 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 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 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-

dicat~~e~~ 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~~s~~ isolated from mycelial and conidial cells.

All of the~~se~~ 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.