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DNA Polymorphism in the Genome: A Raman Spectroscopic Study

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A laser Raman microscope was used to obtain spectra from polytene chromosomes in transcriptionally active salivary gland nuclei of Drosophila melanogaster third-instar larvae. Bands for DNA, nucleosomes, proteins, and nuclear membrane fatty acids were identified in difference spectra generated from 1:1 subtraction of the cytoplasm spectra from nuclear spectra. The presence of bands corresponding to B-, C- and disordered-DNA secondary structure indicates that DNA polymorphism exists within salivary gland cells of D. melanogaster.

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OBJECTIVE: The objective was to observe conformations of DNA in a living cell.

APPROACH: The details of the approach are described in the enclosed preprints.[1, 2] Here a brief overview is appropriate. We approached the objective using Raman spectroscopy. More specifically we implemented this inelastic light scattering technique using an intracavity microscope as described by Patapoff *et al.* [3] The sample we used was the salivary gland of *Drosophila melanogaster.* The gland was extracted at the development stage of the larvae which produced the largest amount of actively transcribing DNA. Several significant problems occurred during the preliminary measurements: 1) sample damage due to incorrect laser frequency, 2) low signal-to -noise ratio of the DNA spectra.

ACCOMPLISHMENTS: Although the interpretation of our divided spectrum is difficult due to the large number of Raman bands which are observed in such a complicated system as the nucleus of a eukaryotic cell we were able to assign a large number of the bands. We eliminated the sample damage problem by switching to a laser which lases in the red. The second problem was circumvented by dividing the spectrum of the nucleus by that of the cytoplasm immediately outside the nucleus.

Many additional accomplishments presented themselves during the period of this grant–as can be seen by inspecting the list of publications with this Office of Naval Research grant number in their Acknowledgments. This situation occurred do to the efficient graduate students in the Laboratory of Professor Warner Peticolas, the many ideas of Professor Peticolas, and to some extent a backlog of research results from the laboratory of the P. I.

Principally the projects which the grant afforded completion of were:

• The completion of a project concerning a refined calculation of highly simplified DNA for far-infrared frequencies by the P. I. in Stuttgart, Germany with Professors Glenn Edwards and Ludwig Genzel. [4] The present work exploits the symbolic manipulation power of *Mathematica* by avoiding approximations necessary with less sophisticated programs.

• The complete writing of the first Physical Review paper on the low frequency vibrational spectroscopy of a drug-DNA complex: netropsin-DNA. Both the Raman and Infrared spectra were published. The conclusion of most interest is the surprising realization that netropsin ef-

fects the DNA largely as a counterion! Several researchers (Prohofsky and Lee) expected much more severe differences in the dynamics of the molecule. In retrospect, this respect could have been predicted by the X-ray work of Dickerson and coworkers [5] since they concluded that the netrospin has only van der Waals, hydrogen bonding, and weak electrostatic interactions with the DNA.

Special note: This paper has had an enormous impact on the PI's institutional standing since it is the first *Physical Review* paper with a Reed student as co-author.

• During the period of the grant a *Biopolymers* paper was written which detailed unusual structures of DNA on two levels: first, the films used in the study showed a dramatic fractal-like crystalline growth pattern in them, and due to this increased crystallintiy the conformation of the DNA within the films was able to maintain many of the characteristics of B form DNA. Some absorption bands such as the 1275 cm^{-1} band of thymine did switch to A conformations, but for the most part the molecule was stabilized in the B conformation at 0 % RH by the increased interhelical interactions due to particular sample preparation. (It does not escape the notice of the PI that forces analogous to such interhelical interactions are bound by intuition to play a major role in biological nanoıabrication.)

• Some ultraviolet resonance Raman measurements and consequent conclusions on bacteriorhodopsin were were detailed in a paper submitted to the *Journal of Raman Spectroscopy.* We used the sensitivity of the Fermi resonance of the tryptophan residues to water activity to

detect the effect of salt on the proton pumping mechanism. Increased salt concentrations induces increased the hydrophobicity of the retinal pocket and thereby alters the charge displacement ability of the proton pump.

SIGNIFICANCE:

The major conclusion of the study focussed on the grant objective is that the DNA in the nucleus is polymorphic, it i. e. the DNA is not only in the B- conformation as the prevailing, *albeit* thinly-supported, dogma would suggest. This conclusion is significant for several reasons. On the technical level we have demonstrated that it is possible to extend the comparison between X-ray crystal diffraction and Raman scattering on a series of DNA crystal to DNA in a living cell. This point brings the proverbial "us" significantly closer to solving clinical problems by understanding DNA conformations in vivo. This technical extension is important because X ray diffraction would seemingly never be useful *in vivo.* NMR is clearly a powerful technique in solution-like environments, and between NMR and vibrational spectroscopy a structural and dynamical picture of DNA under physiological conditions will develop. A powerful extension of this project of great clinical import would be to implant antisense oligonucleotides in the salivary gland and observe the structural transitions. It must be stressed that such experiments are difficult and corroboration is necessary before bombproof conclusions can be incorporated into the lore of spectroscopic data available for explaining biochemical functioning such as transcription and replication.

PUBLICATION AND ABSTRACTS:

•Moenne-Loccoz, P. Powell, J. W., & Peticolas, W. L.,"Counterion-Mediated Hydrophobicity of the Retinal Pocket of Bacteriorhodopsin" submitted to J. Raman Spect., August 1993.

• **Sclavi, B.**, Peticolas, W. L., & Powell, J. W.,"Fractal-like Patterns in DNA Films, B form at O% RH, and Anti-heteronomous DNA: an Infrared Study," Accepted by Biopolymers, Dec. 1993.

•Lee,S. A., **Sclavi, B.**, Powell, J. W., Williamson, W. III, & Rupprecht, A., "Raman and infrared study of the vibrational dynamics of the netropsin-DNA complex" Phys. Rev E., 48, 2240 - 2245 (1993).

•Powell, J. W. and Crandall, R. E., "Lattice Dynamics for a Simplified DNA Model," Mathematica in Education.2 No.3 (Spring 1993).

•T. E. Holland, J. W. Powell, and W. L. Peticolas, Evidence for DNA polymorphsim in vivo: Laser Raman microscopy of polytene chromosomes in Intact Drosopila melanogaster salivary gland cells," Submitted to J. Biomol. Struct. Dynam., Aug, 1993.

•T. Rush III, H. Yong, J. W. Powell, & W. L. Peticolas, "Self-association of dC10 in the pH range 5 - 7 competes with the formation of the double helix dC10dG10 or the triple helix dC10+dC10dG10" Submitted to Biochemistry rapid communications, Sept. 1993; however, rejected. Resubmission planned.

0.1 Contributed Papers

•J.W. POWELL, P. MOENNE-LOCCOZ, & W.L. PETICOLAS, "Counterion-Mediated Hydrophobicity of the Retinal Pocket of Bacteriorhodopsin," accepted, Bull. Am. Phys. 00, 000 (1994).

•J.W. POWELL, T.E. HOLLAND, & W.L. PETICOLAS, "Evidence for DNA polymorphism *In Vivo*: Laser Raman Microscopy of Polytene Chromosomes in Intact *Drosophila melanogaster* Salivary Gland Cells," accepted, Bull. Am. Phys. 00, 000 (1994).

•J.W. Powell & **B. Sclavi**, "Conformational Study of d(GGTATACC) and d(GGUAUACC) and Associated Polynucleotides by Infrared Spectroscopy" Bull. Am. Phys.38, 119 (1993).

•S. A. Lee, **B. Sclavi**, J. W. Powell, W. Williamson III, & A. Rupprecht, "Raman and infrared study of the vibrational dynamics of the netropsin-DNA complex" Bull. Am. Phys. Soc. 38, 119 (1993).

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[1] Powell, J. W., Holland, T. E., & Peticolas, W. L., "Evidence for DNA polymorphism *In Vivo*: Laser Raman Microscopy of Polytene Chromosomes in Intact *Drosophila melanogaster* Salivary Gland Cells," accepted, (1994) *Bull. Am. Phys.* **00**, 000.

[2] Powell, J. W., Holland, T. E., & Peticolas, W. L., "Evidence for DNA polymorphism *In Vivo*: Laser Raman Microscopy of Poly-

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[3] Patapoff, T.W., Thomas, G.A., Wang, Y. & Peticolas, W.L., (1988) *Biopolymers* 27, 493.

[4] Powell, J. W., Edwards, G. S., Genzel, L., Kremer, F., Wittlin, A., Kubasek, W., & W. Peticolas, (1987) *Phys. Rev. A* **35**, 3929.

[5] Kopka, M.L., Pjura, P., Yoon, C., Goodsell, D., & R.E. Dickerson, in *Structure and Motion: Membranes, Nucleic Acids and Proteins*, edited by E. Clementi, G. Corongiu, M.H. Sarma, and R.H. Sarma, (Adenine Press, Guilderland, 1985) 461.