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Single molecule elasticity studies of protein-DNA interactions in the bacterial nucleoid

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The genetic material in bacterial cells is organized in a structure called the nucleoid. In Escherichia coli this nucleoprotein complex consists of a single circular DNA molecule 4.7 million base pairs long, RNA, and a large variety of bound proteins. Among these, about ten so-called histone-like proteins including the Heat Unstable protein (HU), the Integration Host Factor (IHF) and Histone-like Nucleoid Structuring Protein (H-NS) shape the short-scale structure of the nucleoid by bending DNA locally upon binding. These proteins therefore play an important role in compacting the DNA molecule, in addition to other factors such as supercoiling and macromolecular crowding. Not much is known about the large structure of the bacterial nucleoid, and how and by how much each protein contributes to nucleoid compaction.

We tackle these issues by using single molecule techniques, which allow one to proble low-affinity and non-specific protein-DNA interactions. We measure the elastic response of single lambda DNA molecules in solutions of different histone-like proteins of different concentrations. Our setup is illustrated in Fig. 1 where we show a single DNA molecule in solution, tethering a micron-sized paramagnetic bead to a glass substrate. The bead is observed through an optical microscope. A pair of external magnets exerts a force on the bead, thereby inducing tension on the DNA molecule. The tension can be deduced from measuring the average extent of Brownian motion of the bead and using the equipartition theorem of statistical mechanics. The extension of the DNA molecule can in turn be deduced by comparing the instantaneous bead image to a library of images obtained at known heights.

We have carried out systematic experiments with IHF, and present in our poster some of the findings, together with preliminary results on HU-DNA interactions. We illustrate our results in Fig. 2, where we show force extension curves in the case of wild-type IHF at different concentrations: 0 nM IHF (full circles), 250 nM IHF (stars) and 1250 nM IHF (empty triangles). Also shown are the results using an IHF mutant (betaR46C), whose activity in vivo is one hundredth that of wild-type IHF. Lines through the data points are fits to the data using a statistical mechanical model. Our results show that:

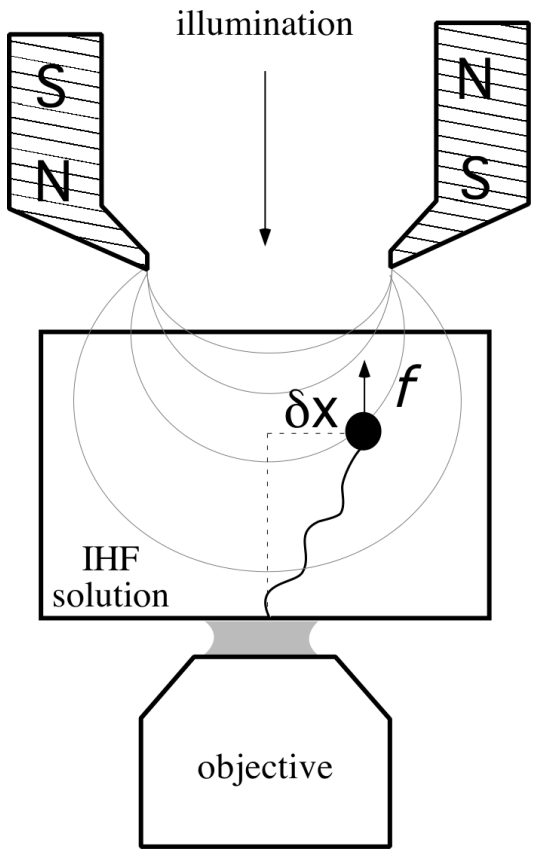

Fig. 1 *Experimental setup for force-extension measurements of protein-DNA complexes: a force is exerted on the DNA tethered paramagnetic bead by a pair of magnets, whose height can be controlled to change the force's magnitude.*

(i) the DNA-IHF complex retains a random, though more compact coiled configuration for zero or small values of the tension.

(ii) IHF induces DNA compaction by binding to multiple DNA sites with low specificity.

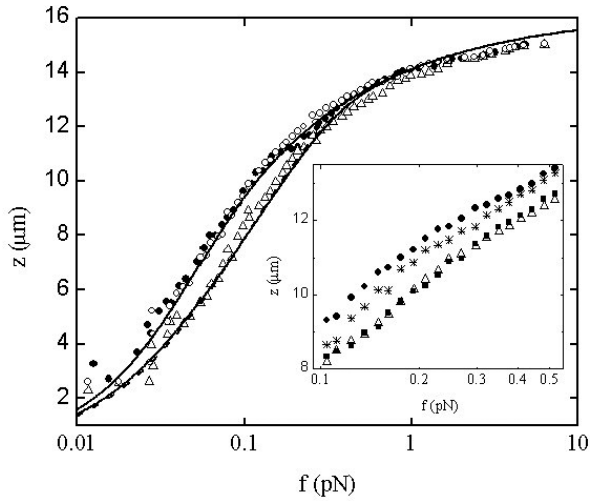

Fig. 2 *Extension z versus force f curves for a lambda phage DNA molecule in solutions of wild-type and mutant IHF of different concentration. Lines are fits with a theoretical model.*

(iii) With increasing tension on the DNA the elastic properties of bare DNA are recovered.

This behavior is consistent with the predictions of a statistical mechanical model describing how proteins bending DNA are driven off by an applied tension on the DNA molecule. Estimates of the amount of bound IHF in DNA-IHF complexes obtained from the model for small values of the tension, in the range of concentrations studied, agree very well with independent measurements of this quantity obtained from the analysis of DNA-IHF cross-linking.

This work was done in collaboration with Opher Gileadi from the Department of Molecular Genetics, Weizmann Institute, and with Amos Oppenheim, Department of Molecular Genetics and Biotechnology, The Hebrew University-Hadassah Medical School.

Selected Publications

Jaffar Ali, B. M., Amit, R., Braslavsky, I., Oppenheim, A. B., Gileadi, O. and Stavans, J. (2001) Proc. Natl. Acad. Sci. USA 98, 10658-10663.

Acknowledgements

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