Study of Thyroid Hormone Receptor/ DNA Interaction

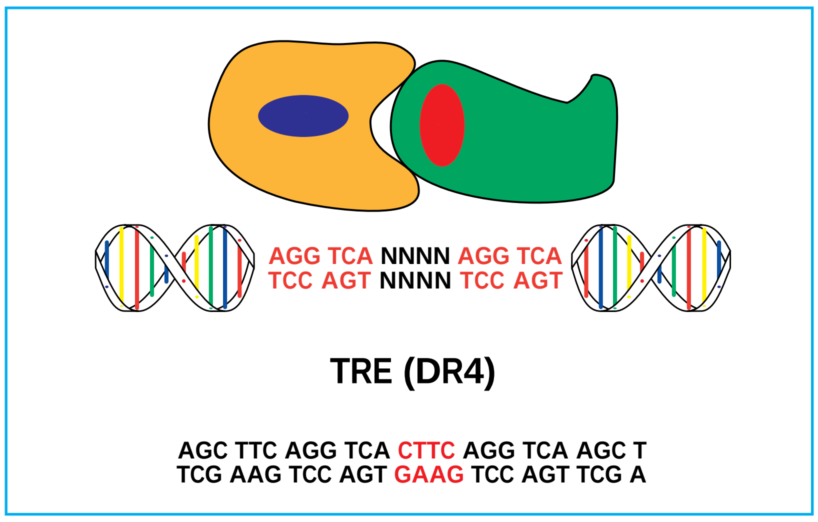

Fig.1: Binding of RXR/TR-heterodimer to thyroid hormone response element.

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INTRODUCTION

The effect of steroid and related hormones is based on their interaction with nuclear receptors. Thyroid hormone receptors (TRs) are ligand-dependent nuclear transcription factors involved in development, differentiation and the physiological response to diverse stimuli. Together with nuclear proteins such as retinoid-X-receptors (RXRs), these molecules (thyroid receptor with its bound cofactor T3) bind as heterodimers to thyroid hormone response elements (TREs) within the promotor region of thyroid hormone (T3) regulated genes.

Fluorescence correlation spectroscopy (FCS) is a new analytical technology which may be used to study biomolecular interactions in biological assays.

Most conventional techniques for the investigation of DNA protein interactions are based on radioactive methods (e.g. filter binding, gel retardation). Both methods perturb the equilibrium of the reaction system. Using FCS, true equilibrium measurements may be carried out in homogeneous solutions which promises to open new dimensions in the understanding of gene regulation.

We have studied thyroid hormone receptor-DNA binding using FCS and in gel retardation experiments.

MATERIALS AND METHODS

Substances: Recombinant heterodimers (RXRα, TRα chicken 100 kDα); oligonucleotides (24 nucleotides, containing the TRE consensus sequence) from NAPS and ABI. One of the oligonucleotide strands was labeled with tetramethyl-rhodamine and the complementary one with ^{32}P; annealing reaction was carried out according to Sambrook and Maniatis; purification by polyacrylamide gel electrophoresis. Buffer: 60 mM KCl, 5 mM MgCl$_2$, 10 mM Hepes, pH 7.5. Reaction: DNA (1nM) was complexed with different amounts of receptor, final volume 20 µl; 40 min. on ice. Measurement: eight chamber coverglasses (Nunc); room temperature; data aquisition time for all experiments 60 s. 10 µl of the solution actually measured were applied directly to a polyacrylamide gel in order to obtain a reliable comparison between the FCS and gel retardation experiments. Equipment: ConfoCor with an helium neon laser (Uniphase, 1,5 mW, 543,5 nm), a C-APCHROMAT 40x/1.2 W objective (Zeiss), and a 580DF30 interference filter (Omega). Analysis: Using the two-component model (FCS ACCESS software package); Phospho Imager (Molecular Devices).

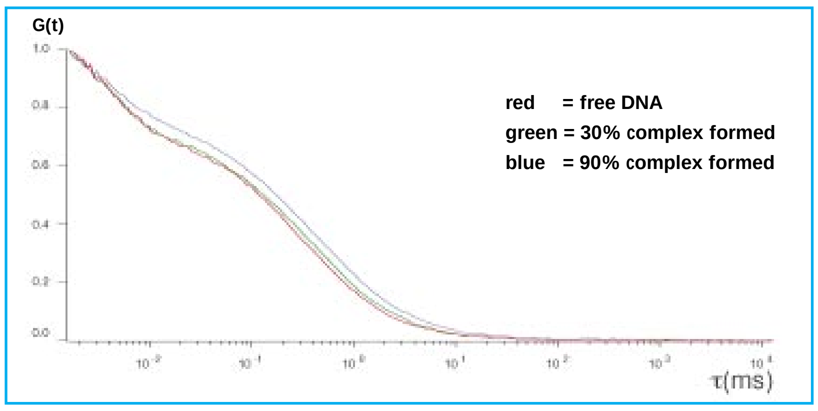

Fig.2: Correlation functions G(t) of different DNA/protein ratios showing increasing amounts of complex formation.

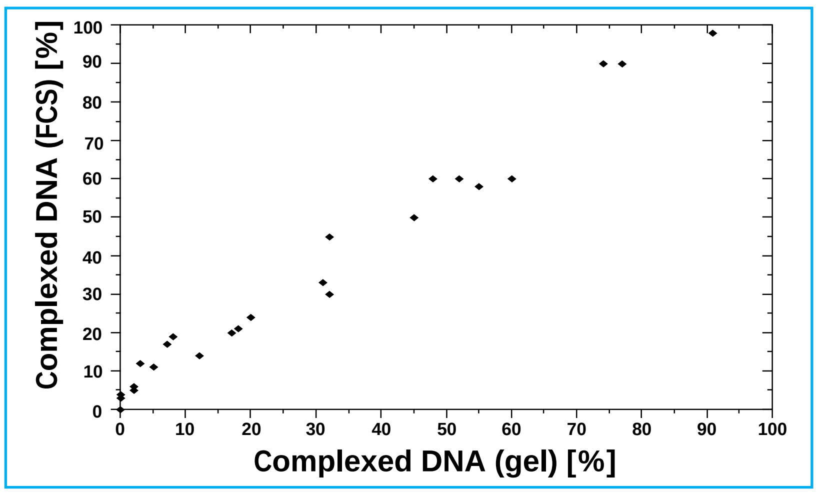

Fig.3: Direct comparison of FCS and gel retardation results. The amount of formed complex obtained by analyzing the FCS-data is plotted against the amount of complex calculated from the gel experiments.

RESULTS

Complexed DNA showed a significantly retarded mobility on the gel compared to free DNA. Increasing amounts of TR/RXR heterodimer added favoured the formation of the DNA-protein complex. Though the difference in diffusion time through the focus of free (345 µs) vs. bound DNA (752 µs) was relatively small, we could reproducibly calculate the percentage of the complex formed (Fig. 2). The results of FCS and gel retardation experiments were in good agreement (Fig. 3). The experiments were performed in a homogenous solution to avoid artifacts arising from surface interactions. Specific DNA-protein interactions require extremely sensitive quantification techniques as they are characterized by high binding constants. In contrast to conventional methods (e.g. gel retardation, filter binding) FCS allows measurement without interfering with the equilibrium. Fig.3 reveals a small, but significantly higher percentage of protein-DNA complexes detected by FCS as compared to gel retardation. By using FCS, the well known problem of complexes dissociating during the run of the gel, if the binding constant is not high enough, is avoided.

LITERATURE

[1] **T. Krietsch,** *PhD. Theses, University Hannover, Germany, 1995*
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SUMMARY

The results of FCS measurements and radioactive gel retardation experiments correlate very well. We have shown that FCS is a highly sensitive method for the quantification of protein-DNA interactions in a homogeneous solution. FCS enables measurements to be performed without disrupting the chemical equilibrium. The measurements are fast, highly reproducible, and eliminate the need for expensive waste disposal and handling of harzardous radioactive material.

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