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A simple procedure for visualising protein – nucleic acid complexes by photochemical crosslinking

Martin Buck* and Wendy Cannon
Agricultural and Food Research Council, Institute of Plant Science Research, Nitrogen Fixation Laboratory, University of Sussex, Brighton BN1 9RQ, UK

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This paper reports a simple and general approach for visualising changes in DNA structure accompanying the crosslinking of protein and nucleic acid by irradiation with ultraviolet light from an inexpensive germicidal lamp. The promiscuous nature of the light-induced reaction makes it generally useful for probing the interface of protein–nucleic acid complexes (1).

In studies on the RNA polymerase sigma factor σ^N (σ^{54}) we have shown by conventional means, including the isolation on denaturing gels of a DNA-σ^N crosslinked complex using oligonucleotides as binding sites, that a specific complex can be formed between σ^N and its target promoter DNA sequence (2,3). To visualise the changes in DNA structure accompanying formation of the crosslinked complex, we generated a short double-stranded target (based on our oligonucleotide crosslinking studies) by hybridising a promoter oligonucleotide to a complementary sequence represented on a single strand DNA template derived from a recombinant M13 phage clone. We crosslinked the σ^N protein to this, removed the protein and oligonucleotide by proteinase K and exonuclease III digestions and then subjected the reacted single-stranded M13-derived DNA to analysis by primer extension. As shown in the figure, σ^N and its holoenzyme are effective at suppressing the UV light reactivity of certain residues in the promoter target DNA, probably by inhibition of pyrimidine dimer formation.

The single-stranded template is a very efficient template for primer extension, and the use of a limited double-stranded region in the binding assay effectively edits out the formation of complexes at non-specific sites. Conditions for crosslinking of the protein of interest can be conveniently established with short probes (1) and then extended to the type of analysis described here to visualise changes to individual bases within the binding site. Subsequently more refined time-resolved and laser light-induced studies may be performed (4,5,6). Furthermore, the inclusion of a synthetic DNA strand in the binding site allows the ready substitution of the usual four DNA bases with analogues or the generation of mismatched or looped regions to evaluate their influence upon protein binding, for example synthetic transcription 'bubbles'.

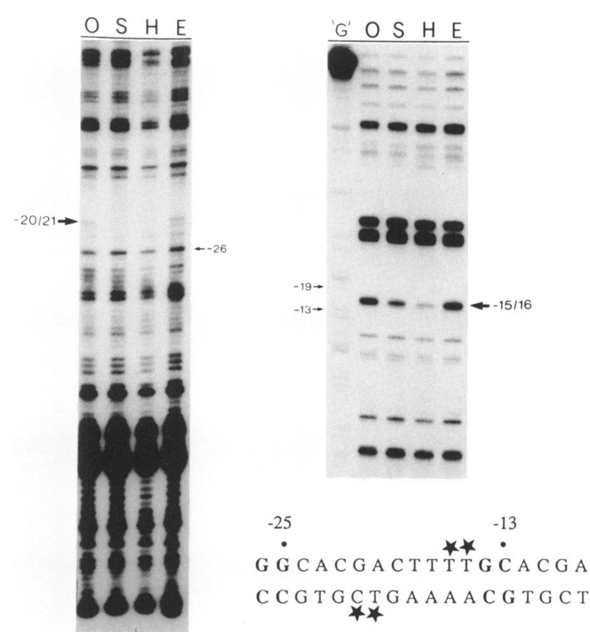

Figure 1. Binding of σ^N and its holoenzyme to promoter sequences detected by ultraviolet light-induced complex formation and primer extension analysis. For assay, template DNA was prepared by hybridising a five-fold excess of 32 base oligonucleotide from −35 to −4 to single strand recombinant M13mp19 or mp18 DNA carrying the *Rhizobium meliloti nifH* promoter sequence (3). This (0.2 pmoles) was incubated in a 20 μl reaction with no protein (0), 2.5 μM σ^N (S), 500 nM holoenzyme (H) or 600 nM core polymerase (E) as described previously (3) and irradiated on ice for 20'with a 254 nm light source. After irradiation the reaction solution was made 0.02% (w/v) in SDS and 5 μg of proteinase K was added for one hour at 37°C. The sample was then phenol-extracted twice and the nucleic acids recovered by ethanol precipitation. The DNA was resuspended in 20 μl of binding buffer and incubated with 100 units of exonuclease III for 30' at 37°C. The reaction was terminated by phenol extraction, DNA ethanol-precipitated and finally redissolved in 10 μl of $T_{10}E_{0.1}$, pH 8.0. To 5 μl of this solution 0.2 pmoles of universal sequencing primer 5' end-labelled (1,500 Ci/mMol) was added to give a final volume of 10 μl in 15 mM Tris–HCl, 10 mM $MgCl_2$, pH 8.0. The primer was annealed by heating at 60°C for 15' in a covered block, cooling slowly and extended by the addition of Klenow DNA polymerase (1 μl, 1 unit) and dNTPs (1 μl of 2.5 mM) for 30' at 37°C. Products (25% of the sample) were analysed on 6% sequencing gels, with chemical sequencing 'G' ladders as markers. Autoradiography was at −70°C for 10–72 hours with screens. The reactivity of bases at −20, 21 and −15, −16 is suppressed by protein binding, these are indicated within the *R.meliloti* σ^N recognition sequence. Sigma-N and its holoenzyme bind specifically, core polymerase non-specifically, to promoter DNA (2,3).

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* To whom correspondence should be addressed

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