	Experiment title: **Movement of the RNA-polymerase on the DNA-template: A fast OH•- radical footprinting study using short pulses of OH• produced by synchrotron radiation**	**Experiment number**: LS-1853
Beamline: ID9	**Date of experiment**: from: 8.June 2001 to: 12 June 2001	**Date of report**: 1.Sept.2001
Shifts: 9	**Local contact(s)**: Michael Wulff	*Received at ESRF:*

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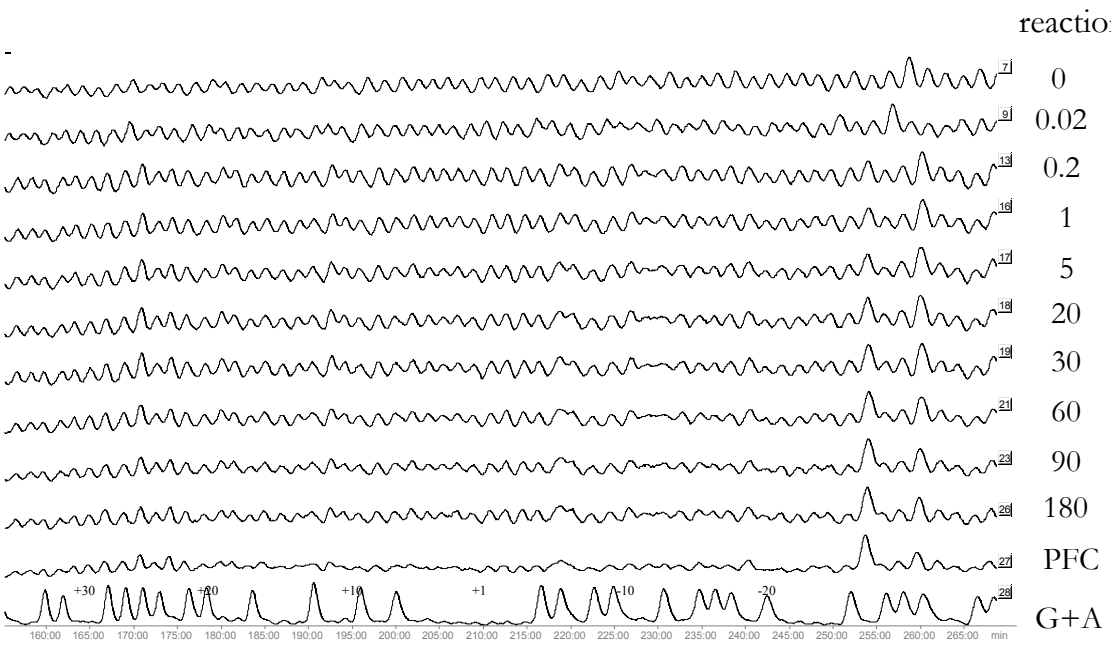
Report:

Introduction:

Previous studies have shown that DNA is cleaved by x-ray induced OH-radicals after exposure of 2msec using the white beam of ID9. Based on the results of these studies, we were able to achieve the goal of our last proposal LS-1853, namely to follow the kinetics of RNA polymerase binding to DNA.
In order to comply with ESRF safety requirements, the radioactive labelling of DNA was replaced by a fluorescence labelling technique. This technique had the additional advantage that quantification of the band intensity was greatly facilitated.
Fig.1 shows the result of a time dependent x-ray induced cleavage pattern of DNA after mixing with *E.coli* RNA polymerase using the new fluorescence technique.

Fig.1



Experimenal conditions:

A 157 base pair double stranded DNA containing the promoter A1 of the phage T7 was mixed with E.*coli* RNA polymerase by means of a stopped flow apparatus. Reaction of RNA polymerase and DNA was allowed for the time intervals indicated in Fig. 1. Subsequently all samples were exposed to the white ID9 beam for 2msec, permitting OH-radical cleavage. The DNA cleavage products were electrophoretically separated and the fluorescent labelled bands were detected as peaks, as shown in Fig.1.

Controls:

The lane G+A is a sequence standard which permits determination of the base positions. Base position +1 refers to the starting point of RNA synthesis. The region above base position +26 was used to normalize the cleavage patterns, since no RNA polymerase binding occurs in this region, as previous chemical footprinting data have shown. The lane "PFC" is the footprint of a complex preformed for 15 min. It is identical with that in lane "180", indicating that the reaction is finished after 180 sec. Lane "0" shows the cleavage pattern DNA without RNA polymerase.

Interpretation:

In collaboration with Bianca Sclavi the cleavage pattern was quantitatively evaluated. The extent of cleavage obtained at different reaction intervals shown in Fig 1 was compared at different base positions (vertical comparison of the peaks in Fig. 1). Fig. 2 shows the extent of cleavage at the base positions indicated. The different colours of the histogram show the extent of cleavage after the different reaction time intervals indicated in the legend.

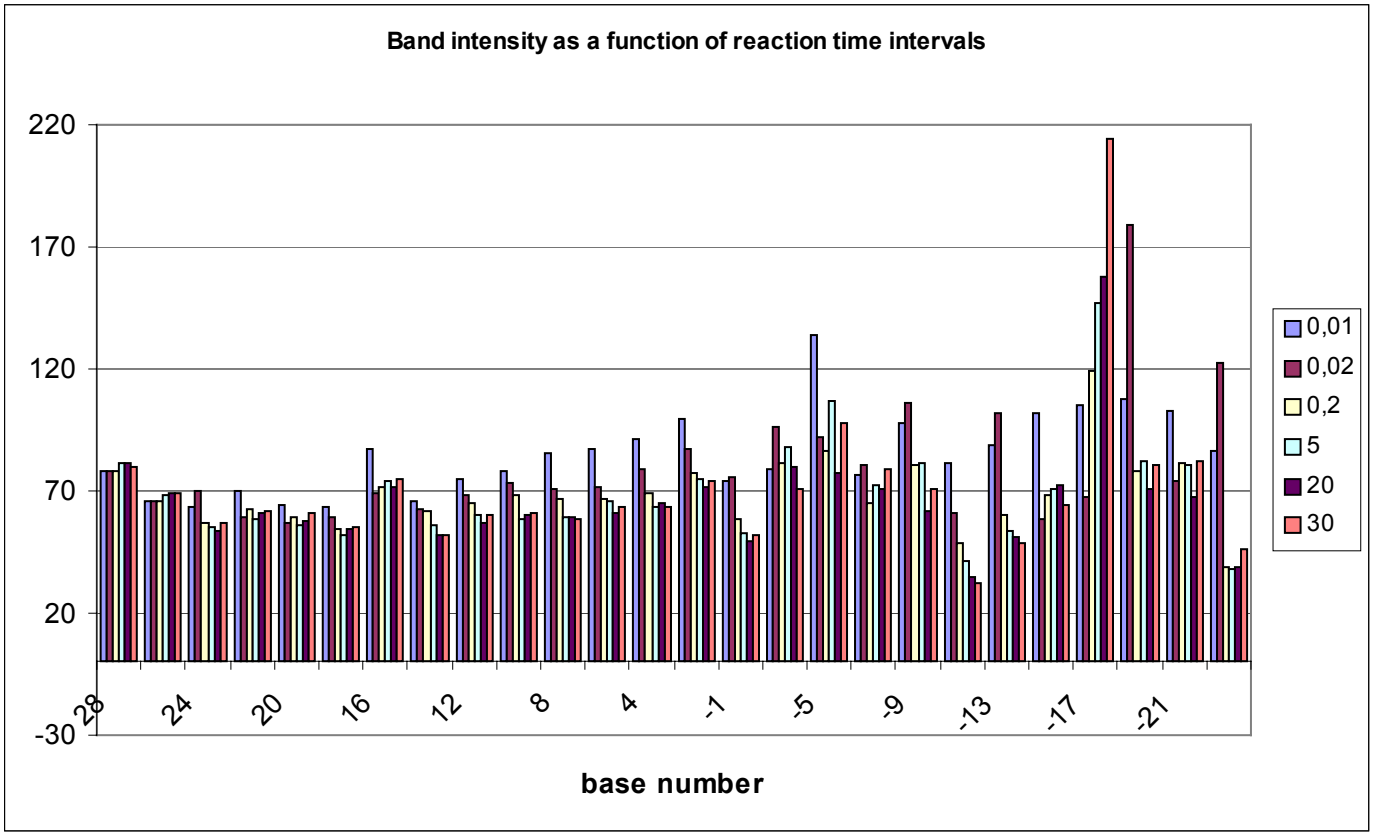


Fig. 2 shows that the cleavage pattern in the region above +20 does not change significantly. This is in line with results from classical footprinting studies using the Fenton reaction, since RNA polymerase binds between base position +18 and –40.

Most interesting and surprising is the finding that the bases located within the region interacting with RNA polymeras (base position +16 to –21) show rather individual time-dependent response to the OH radicals. For example, the bases located in the region –16 to –5 show enhanced cleavage after 10 msec reaction and after longer reaction intervals protection (as expected). The base at position –17 shows an enhancement of cleavage. We do not fully understand this behaviour, but it indicates –this is our interpretation- differences in the time dependent interaction of RNA polymerase with each of the DNA bases. This is a rather spectacular result, since it allows us to follow the interaction of RNA polymerase with promoter DNA on a single base level. Verifying this new finding will be the main object of our next proposal.