The Decondensation factor 31 specifically binds to ssRNA but not to ssDNA or dsDNA

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Abstract

Histone proteins are associated with DNA and form a nucleoprotein structure termed chromatin. The chromatin structure allows the eukaryotic genome to be stored and transported but also needs to be accessible to various crucial DNA dependent processes Thus, histone modifying, DNA modifying and chromatin binding proteins specifically interact and regulate chromatin.. Interestingly, many of the chromatin modifying proteins also display RNA binding capacity. Here we characterize the specific interaction of the Decondensation factor 31 (Df31) with nucleic acids. Df31 was recently shown to form be part of a RNA dependent regulating mechanism influencing chromatin structure and function.

Introduction

In eukaryotic cells the DNA is packed into chromatin by the association of histone molecules and non-histone proteins. Various non-histone proteins influence the structure of chromatin and thus also the function of chromatin. A class of proteins called chromatin remodelling complexes are capable to move nucleosomes along the DNA, thereby revealing or hiding transcription factor binding sites. Other protein classes change the functionality of chromatin by the modification of chromatin with epigenetic marks like methylation or acetylation. Interestingly, many of these proteins involved in chromatin remodelling and modification have the capacity to bind RNA directly or interact with complexes that contain RNA (Mattick et al., 2009). The binding of HP1, a major structural component of heterochromatin, for example, is highly dependent on the presence of RNA (Maison et al., 2002).

Moreover, DNMTs and DNA methyl binding proteins recognize and interact with RNA (Jeffery and Nakielny, 2004). Interestingly, only a few of these chromatin remodelling proteins are described to possess a preference for particular DNA sequences. However the modifications have to be purposefully directed to “different positions, in different loci, in different cells” (Mattick et al., 2009), implying another layer of information to guide these processes, which seem to be RNA based.
In a recent study the *Drosophila* Decondensation factor 31 (Df31) was shown to be involved in an RNA dependent mechanism regulating chromatin compaction (Schubert *et al.*, 2012). To determine whether Df31 is capable to bind nucleic acids and especially RNA MicroScale Thermophoresis analyses were performed.

Results

In this application note, the binding behavior of Df31 to nucleic acids was evaluated, using MicroScale Thermophoresis.
Fam or Cy5-labeled random ssRNA, ssDNA, dsDNA molecules of same sequence and length (29 nt) were used in a concentration of 50 nM in the experiments.
A serial dilution of Df31 was added with starting concentration of 680 µM to the corresponding nucleic acid. The samples were filled into standard capillaries and incubated at 27 °C for 15 min, prior to the MST measurement.
For the assay three independent measurements were performed. A clear binding curve for the interaction of the Df31 protein and ssRNA could be detected. However, ssDNA and dsDNA showed no binding to Df31.

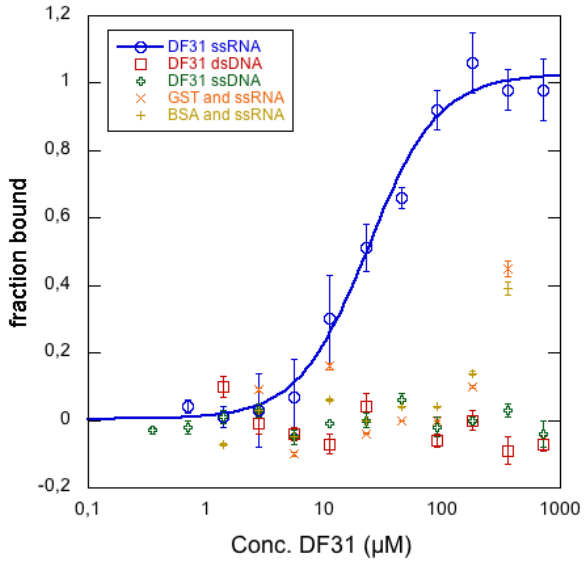

Fig. 1 Measurement of Df31 binding to nucleic acids. MST values were normalized to fraction bound. 3 independent measurements where performed to obtain the depicted binding curve. The K_d of the Df31-ssRNA interaction was 24.01 ± 3.15 µM.

The calculated K_d from the measurements of the Df31 binding to ssRNA was 24.01 ± 3.15 µM.
As control we also measured the interaction of BSA and GST with our target ssRNA. The experiment was performed as described for the Df31 protein. No interaction could be detected.

Conclusion

The study provides an example that MicroScale Thermophoresis is capable of measuring and detecting specific interactions between nucleic acids and their interacting proteins. Straightforward control experiments proof the specificity of the interaction. Experiments are easily setup and affinities can be determined in a timely manner.

Material and Methods

Assay conditions
For the experiment random FAM or Cy5-labeled ssRNA, ssDNA and dsDNA molecules of same sequence and length were used at the concentration of 50 nM. Unlabeled Df31 was added in 1:1 dilutions beginning at 680 µM. Samples were prepared in a buffer containing 20 mM Tris-HCl pH 7.4, 1.5 mM $MgCl_2$, 0.5 mM EGTA, 200 mM KCl, 10 % Glycerol and 0.05 (v/v) % NP-40. For the measurement the samples were filled into standard capillaries.

Instrumentation
The measurements were performed on a NanoTemper Monolith NT.115 instrument.
The measurement was performed in standard capillaries at 30 % LED and 30 % MST power with Laser-On time 30 sec and Laser-Off time 5 sec.

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