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Brief report

Influence of the live cell DNA marker DRAQ5 on chromatin-associated processes

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ABSTRACT

In the last decade, live cell fluorescence microscopy experiments have revolutionized cellular and molecular biology, enabling the localization of proteins within cellular compartments to be analysed and to determine kinetic parameters of enzymatic reactions in living nuclei to be measured. Recently, *in vivo* DNA labelling by DNA-stains such as DRAQ5, has provided the opportunity to measure kinetic reactions of GFP-fused proteins in targeted areas of the nucleus with different chromatin compaction levels. To verify the suitability of combining DRAQ5-staining with protein dynamic measurements, we have tested the cellular consequences of DRAQ5 DNA intercalation. We show that DRAQ5 intercalation rapidly modifies both the localization and the mobility properties of several DNA-binding proteins such as histones, DNA repair, replication and transcription factors, by stimulating a release of these proteins from their substrate. Most importantly, the effect of DRAQ5 on the mobility of essential cellular enzymes results in a potent inhibition of the corresponding cellular functions. From these observations, we suggest that great caution must be used when interpreting live cell data obtained using DRAQ5.

1. Introduction

Recently, tools have become available that allow the analysis of molecular mechanisms within living cells. Next to technical developments in microscopy these studies are mainly boosted by the availability of a still increasing set of fluorescent markers, including (genetic) tagging of proteins with green fluorescent protein (GFP) and its many spectral variants and fluorescent probes that target specific sub-cellular components or structures. These tools have enabled the study of *in vivo* cellular localization, dynamic behaviour and interactions of proteins with their partners [1–8]. Particularly, spatio-temporal dynamics studies on DNA-related processes, such as replication, transcription and DNA repair would be greatly aided by viable stains that would differentiate chromatin compaction, since regulation of these processes is tightly linked to chromatin condensation and DNA conformation [9]. The anthraquinone derivate DRAQ5 [10,11] has been recently introduced as a new way to label and visualize DNA and chromatin. This new DNA-probe possesses several advantages for visualizing DNA in living cells [12] compared to other DNA dyes such as 4′,6-diamidino-2-phenylindole (DAPI) and Hoechst 33258, mainly because its deep red $Ex_{\lambda\,max}/Em_{\lambda\,max}$ ($Ex_{\lambda\,max}$ 646 nm; $Em_{\lambda\,max}$ 681; $Em_{\lambda\,range}$ 665 → 800 nm) does not interfere with other fluorescent species used for tagging proteins and at the same time avoids the use of toxic UV irradiation as excitation source [13,14]. Additionally, DRAQ5 penetrates very quickly into the nucleus and stains the DNA stochiometrically without apparent severe cytotoxic effects [13,15]. Finally, DRAQ5 is not bleached under standard imaging conditions and is still clearly detectable 24 h after its addition to cells [12]. These properties make DRAQ5 a suitable DNA dye to be used in live cell imaging and could potentially be coupled to kinetic measurements of cellular activities. However, because DRAQ5 intercalates into the DNA-helix and thereby possibly distorts the helical structure of it, we have investigated the effect of DRAQ5 on DNA/chromatin bound proteins and nuclear processes. Our study shows that DRAQ5 alters the chromatin structure and influences the interaction of a large spectrum of DNA-binding proteins, leading to inhibition of different DNA-dependent processes such as transcription, replication and nucleotide excision repair (NER).

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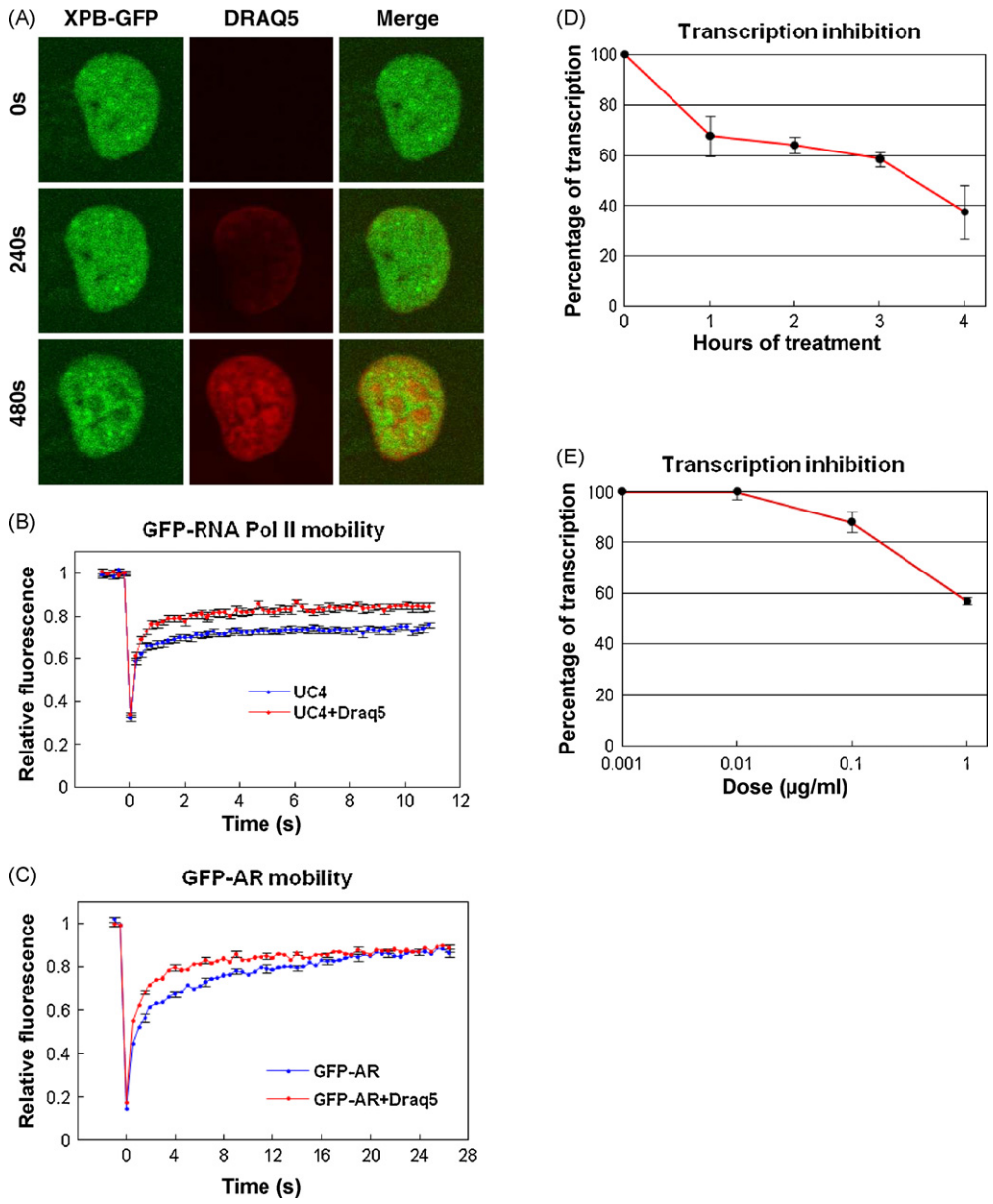

Fig. 1. DRAQ5 DNA intercalation inhibits transcription. (A) Confocal imaging of XPB-GFP expressing fibroblasts after DRAQ5 treatment. Localization of XPB (GFP signal in green) is altered when DRAQ5 (red) is intercalated into the DNA. (B) FRAP analysis of GFP-RNA Pol II in the nucleus of cells incubated (red filled squares) or not (empty blue circle) with DRAQ5 for 1 h. (C) FRAP analysis of GFP-AR in the nucleus of cells incubated (red filled squares) or not (empty blue circle) with DRAQ5 for 1 h. (D) MRC5SV40 transformed human fibroblasts treated with DRAQ5 (5 μM) for different times. Transcription is detected by [^{3}H]uridine incorporation as described in Section 4. (E) Transcription inhibition assay. MRC5SV40 transformed human fibroblasts treated with three different concentrations of DRAQ5 for 3 h. Transcription is detected by [^{3}H]uridine incorporation as described in Section 4.

2. Results and discussion

2.1. Effect of DRAQ5 DNA intercalation on transcription

We tested the effect of DRAQ5 DNA intercalation on cellular transcription by studying differences in localization and/or mobility of the basal transcription factor, TFIIH [16], RNA polymerase II [17] and a specific transcription activator, the androgen receptor [18].

TFIIH is a heterodecameric complex involved in both transcription and nucleotide excision repair. Mutations in some of the subunits can give rise to the genetic disorder xeroderma pigmentosum (XP) and related conditions. TFIIH dynamically interacts with both RNA polymerases I (RNAPI) and II (RNAPII) transcription sites and its dynamic properties as well as its sub-nuclear distribution are greatly influenced by transcription interfering molecules [16]. To investigate whether DRAQ5 has an effect on the interaction of TFIIH with DNA, we used a cell line that stably expresses a biological functional GFP-tagged XPB protein [16] (details on cell line used and expression are in M & M's or simply referred). XPB encodes the largest subunit of the transcription/DNA repair complex TFIIH.

Imaging of untreated cells showed that XPB-GFP is homogenously distributed in the nuclear compartment [16,19]. However, rapidly after treatment with DRAQ5, the sub-cellular localization of TFIIH is substantially changed (Fig. 1A): as DRAQ5 DNA intercalation progressed, TFIIH became more heterogeneously distributed and progressively excluded from the nucleoli.

This result prompted us to test whether this change in localization of a transcription initiation factor would affect the kinetic behaviour of elongation factors as well. Therefore, we