G-quadruplexes unfolding by RHAU helicase

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Abstract

G-quadruplexes (G4) are RNA and DNA secondary structures formed by the stacking of guanine quartets in guanine rich sequences. Quadruplex-prone motifs may be found in key genomic regions such as telomeres, ribosomal DNA, transcriptional activators and regulators or oncogene promoters. A number of proteins involved in various biological processes are able to interact with G4s. Among them, proteins dedicated to nucleic acids unwinding such as WRN, BLM, FANCJ or PIF1, can unfold G4 structures. Mutations of these helicases are linked to genome instability and to increases in cancer risks. Here, we present a high-throughput fluorescence-based reliable, inexpensive and fast assay to study G4/RHAU interaction. RHAU is an RNA helicase known as the major source of G4 resolution in HeLa cells. Our assay allows to monitor the unfolding properties of RHAU towards DNA and RNA quadruplexes in parallel and to screen for the optimal conditions for its activity.

Keywords

G-quadruplex; Helicase; RHAU; G4R1; DHX36; G4 ligand; helicase assay

1. Introduction

G-quadruplexes (G4) are DNA and RNA nucleic acid secondary structures formed by guanine-rich strands, where four guanines associate through Hoogsteen hydrogen bonds, resulting in planar quartets which are stacked on each other. Computational studies searching for a G4-prone motif showed that, in the human genome, sequences highly prone to adopt a G4 conformation are found in key regions such as telomeres, oncogene promoters or mRNA UTR.[1–3] Given their location, they are probably involved in numerous biological processes ranging from regulation of gene expression to aging. [4,5] This hypothesis was reinforced by their direct visualisation using ligand or structure-specific antibodies and their interactions with numerous proteins involved in such biological processes.[6–9]

Helicases are motor proteins able to unwind nucleic acids. They are divided into two major groups according to the nature of their substrate: DNA or RNA helicases. The majority of RNA helicases belong mostly to two main subgroups depending on the amino acid composition of their helicase domain: the DEAD and DEAH box families. RHAU (for *RNA helicase associated with AU rich element*) is the gene product of Dhx36 (also named Mlel1 or G4R1) and belongs to the DEAH-box helicase family.[10]

RHAU exists under two isoforms: an abundant nuclear one and a shorter cytoplasmic one (with 14 amino acids missing in the helicase domain). [11,12] The full-length RHAU nuclear isoform is 1008 amino acid-long and has a molecular weight of 115 kDa. Its structure can be divided into three major parts: an N-terminal domain (≈ 200 amino acids), a central helicase core (≈ 400 amino acids) and a C-terminal domain (≈ 400 amino acids). Its helicase domain comprises domains enabling both ATP binding and hydrolysis, nucleic acids binding and unwinding, in addition to a nuclear export signal.[10,12]

In contrary to the C-terminal domain, RHAU N-terminal domain is well documented in the literature. It comprises on its first 105 amino acid residues an RNA-binding stretch required for its recruitment to stress granules,[13] containing a glycine rich region with a RGG-box motif and the RSM (*RHAU specific motif*), a conserved 13 aa-long region from residues 54 to 66 required for G-quadruplex recognition and interaction.[14] A 53 amino acids-long peptide (from residues 53 to 105) within the N-terminal domain comprising the RSM was shown to be sufficient to interact with human telomerase RNA and its DNA equivalent, which both adopt an intramolecular quadruplex conformation.[15] It was also demonstrated that, within this peptide, four amino acids among the most conserved ones (glycine 9, isoleucine 12, glycine

13 and alanine 17) contact the terminal-quartet of the DNA G-quadruplex-forming sequence T95-2T.[16]

RHAU function is still unclear, but it was shown that this protein is overexpressed in breast cancer cell lines,[17] has a role in gene expression[11,12] in addition to its involvement in several other biological processes such as spermatogenesis,[18,19] heart development,[20] mammalian haematopoiesis[21] and regulation of telomere length.[22] As RHAU was identified through its ability to resolve G-quadruplexes and was from that time also known as G-quadruplex Resolvase 1 (G4R1),[23,24] some of the above mentioned RHAU functions are possibly linked to its interaction with G-quadruplex-forming sequences. In fact RHAU was shown to interact with numerous relevant G4 forming sequences such as the *c-myc* oncogene, the promoter region of the transcription factors YY1, the 3'UTR of the transcription factor PITX1 and hTERC (also called hTR) the human telomerase RNA component.[17,22,25–27]

The RHAU full length protein displays higher affinity for a G4 substrate as compared to the N-terminal domain only.[14] Indeed, RHAU unwinding activity depends on the presence of its helicase core while this domain is not required for binding. In fact, it was demonstrated that RHAU N-terminal truncation, bearing the RSM, is necessary and sufficient to bind to G-quadruplexes.[27]

In addition, its affinity is higher for a given G4 RNA-forming sequence as compared to its G4 DNA equivalent.[15,28] According to these results, even if the RHAU N-terminal domain is displaying some activity, complementary activities may be brought by the rest of the protein. In addition, to the best of our knowledge and with the exception of a study carried out using single molecule FRET, only gel experiments have been used to characterize the G4/full-length RHAU interactions.[17,25,28,29,24,30]

In the present work, we use a high-throughput real-time fluorescent G4-based helicase assay, initially developed for the *yeast Saccharomyces cerevisiae* Pif1 helicase, to characterize G4/RHAU interaction.[31] We demonstrate that RHAU is active in comparable conditions as Pif1 but unlike it, RHAU activity towards a G4 substrate such as the *c-myc* oncogene promoter sequence is not sensitive to changes in potassium concentration. We also reveal that RHAU can interact with both G4 and non-G4 substrates such as duplexes. Finally, we provide evidence that the presence of a potent G4 ligand, such as Phen DC3, can limit its unwinding activity towards G-quadruplex substrates.

2. Material and Methods

2.1. RHAU protein

The recombinant nuclear isoform of human RHAU was overexpressed in Baculovirus and purified to homogeneity by Proteogenix (Schiltigheim, France). The purified RHAU enzyme was then aliquoted at 8.7 µM and stored at -80 °C in 20 mM HEPES-KOH buffer (pH 7.7) supplemented by 50 mM KCl, 0.01% Nonidet P-40, 0.5 mM EDTA, 5 mM DTT, 2 mM AEBSF and 10% glycerol. For helicase assays RHAU was further diluted to 100 nM in the same buffer and directly added to the DNA solution. To assess the impact of the potassium concentration on its unwinding activity, the enzyme was also diluted in two other enzyme buffers: *(i)* where KOH was replaced by LiOH and *(ii)* where KOH was replaced by LiOH and KCl by NaCl.

2.2. Oligonucleotides and compounds

Synthetic oligonucleotides used in this study were purchased from Eurogentec (Seraing, Belgium). Stock solutions were prepared into water at concentrations between 100 and 200 μM and stored at -20 °C. Strand concentrations were determined by absorbance at 260 nm using the molar coefficient extinction provided by the manufacturer. The sequences used are summarized in **Table I**. Before all experiments, the oligonucleotides were folded into "systems": we prepared a mixture of Dabcyl-labelled oligonucleotide (1 μM) and FAM-labelled oligonucleotide (0.85 μM) in 20 mM Tris-HCl buffer (pH 7.3) containing 5 mM $MgCl_2$ supplemented by monovalent cations (100 mM NaCl or 1 mM KCl and 99 mM NaCl or 50 mM KCl and 50 mM NaCl or 100 mM KCl). Each mixture was heated for 5 min at 90 °C and then slowly cooled to room temperature. A ratio of 0.85 between the FAM-labeled strand and the Dabcyl-labeled one was required in order to ensure a maximal quenching of the fluorescent signal. Then, each annealed system includes three parts: an 11 nt overhang (either at 5' or 3'-end), a central variable core containing a DNA or RNA G4 structure (alternatively a single-strand or a duplex as controls), and a 15 bp labeled duplex (at the 3' or 5'-end respectively) containing the FAM-Dabcyl fluorophore pair. Systems were stored at -20 °C before use (**Figure 1).**

The G4 ligands used in this study were either Phen DC3 or NMM; they were purchased from Polysciences and Frontier Scientific, respectively. Stock solutions were prepared in DMSO at 1 mM and stored at -20 °C. Before use, intermediate concentration was made at 1 μM in the reaction buffer used.

2.3 The G4 based helicase assay

G4 unfolding reactions were carried out at least in triplicate in 96-well plates (Greiner Bio-one; 96-well, black, flat bottom) at 25 °C and fluorescence was monitored in a microplate reader (Tecan Infinite M1000 PRO). Every replicate contained a 50 µl solution of 10 nM FAM-Dabcyl-labeled system previously annealed at 1 µM, 50 nM of Trap sequence (unlabeled oligonucleotide complementary to the FAM-labeled strand) and the indicated amount of RHAU enzyme. Unfolding reaction was initiated upon ATP addition (5 mM).

The 96-well plate was stirred for 10 s and the FAM fluorescence emission recorded every 10 s using an excitation wavelength of 492 nm and collecting emission at 520 nm. Once the maximum emission was reached with a stable signal (30–45 min), the reaction was stopped and 50 nM of an unlabeled DNA strand complementary to the Dabcyl-labeled G4 bearing strand was added to the reaction well. Plates were then stirred again for 10 s, and emission monitored every 10 s until the maximum of fluorescence emission was reached. The G4-fraction unfolded by RHAU was given by the fluorescence value obtained before addition of the complementary DNA sequence. In the presence of G4-ligands the same procedure was applied but at the beginning of the experiment every replicate contained a 125 nM addition of either Phen DC3 or NMM in each well.

To determine the percentage of systems unwound in solution, we compared the fluorescence emission obtained after ATP addition (which initiates the helicase activity) with the fluorescence emission obtained after addition of the Dabcyl complementary strand (which is supposed to unwind by a kinetic effect all the systems in solution).

3. Results

RHAU activity using the tunable standard fluorescent G4-based helicase assay

In order to characterize RHAU-nucleic acids interactions and particularly its activity towards G-quadruplex structures, we chose to adapt the previously developed 96-well plate fluorescent G4-based helicase assay [28]. This high-throughput assay allows to monitor at the same time the helicase activity of protein towards various nucleic-acid targets in various conditions. This assay is based on monitoring the fluorescence emission of a nucleic acid system labelled with a fluorophore pair: FAM and Dabcyl. It enables energy transfer and allows the estimation by this way of the folded or unfolded state of the system.

First, to test the feasibility of this method, we used a previously designed nucleic acid-system (**Figure 1),** under identical salt conditions.[31] To assess RHAU unwinding activity and confirm its directionality in our conditions we used a mutated *c-myc* oncogene promoter sequence as a control, which adopts a single-stranded conformation, associated with a 15 bp FAM-Dabcyl doubly labelled-duplex and with either a 5' or 3' overhang. As for most of the helicases listed, energy driven from triphosphate nucleoside hydrolysis is required for RHAU unwinding activity.

Our results showed that, unlike ScPif1 helicase, RHAU failed to unwind 5' to 3' c-myc mutated system as demonstrated by the very low rate of systems unwound in solution ([31], **Figure 2**). In contrast, the system bearing a 3' overhang was very efficiently unwound by RHAU in our conditions (over 80% of systems unwound). This confirms the 3' to 5' directionality of RHAU on this kind of substrate and enables us to use the fluorescent G4-based helicase assay to characterize more in detail RHAU-nucleic acids interactions.

Then, we assessed RHAU unwinding activity towards a nucleic acid system with a 3' overhang bearing the G4 DNA structure formed by the c-myc sequence. Our results show that RHAU is able to unfold both the mutated and the G4 c-myc sequence bearing a 3' overhang (**Figure 3**). However, we revealed a slower unfolding process and a lower level of G4 system unwound by RHAU as compared to the mutated system (bearing a single-strand instead of G4 structure on its variable central part) in our conditions. Indeed, RHAU comprises within its N-terminal domain the RSM, which is dedicated to G-quadruplex interaction and a previous study has shown that RHAU was more efficient towards G-quadruplexes as compared to others substrates, including duplexes. [24]

RHAU sensitivity to experimental conditions

It is well-established that monovalent cations play a pivotal role on G-quadruplex stability. A ranking was established regarding their ability to stabilise G-quadruplex structures. Among usual monocations, potassium is the most effective.[32]

We have previously demonstrated using ScPif1 that conditions aimed to increase the thermal stability of a G4 substrate such as a higher potassium concentration, decrease the unwinding ability of the enzyme. Indeed, we observed that a reaction buffer enriched with KCl (100 mM instead of 1 mM KCl with 99 mM NaCl) needs higher amounts of enzyme to ensure the same level of unwinding of G-quadruplexes.[31] As in our conditions RHAU processes the mutated system more easily than the G4-containing system, we tried to check if this preference was dependent on the experimental conditions. To this aim, we modulated the salt conditions by decreasing the potassium concentration. In our conditions, two sources of potassium ions were possible: the reaction buffer which contained only 1 mM KCl (and 99 mM NaCl) and the enzyme buffer, which can contribute to up to 10% of the final potassium concentration.

We tried different salt conditions summarized in supplementary **Table SI** to asses RHAU unwinding activity towards both the mutated and the c-myc bearing system by modulating the potassium contents of the enzyme buffer or the reaction buffer. Our results have shown that the amount of mutated system unwound is not impacted whatever the saline conditions, while the unwinding of the G4 system is slightly impacted by the potassium concentration in the RHAU storage buffer (supplementary **Figure S1A**). Our results have also shown that, regardless of the reaction buffer used, the amount of systems unwound in solution by RHAU is similar in all conditions for both systems (supplementary **Figure S1B**).

These observations contrast with what was expected with the G4 system. Indeed, we postulated that a decrease in potassium concentration would increase the G4 unwinding ability of RHAU due to the decrease of the G4 stability. Owing to these results, we decided to use a reaction buffer supplemented by 50 mM KCl and 50 mM NaCl (in addition to the main RHAU buffer containing KOH and KCl) for the following experiments.

In order to further characterize the G4/ RHAU interaction, we studied the influence of the enzyme:substrate ratio parameter. We assessed RHAU helicase activity using increasing amounts of the enzyme while keeping a constant substrate concentration. As shown in **Figure 4**, increasing RHAU concentration first induced an increase in the percentage of systems

unfolded, before reaching a plateau. However, for the same enzyme:substrate ratio, the proportion of systems unwound is higher for the mutated system. As we were ensured to reach a maximal efficiency for both systems for 1 equivalent of enzyme, we chose this condition for the following experiments.

RHAU activity towards different nucleic acid targets

We decided to add a new control bearing either a DNA or a RNA duplex instead of a single-strand as a central part (**Figure 1)**. Indeed, as RHAU comprises a RNA binding motif, we wanted to compare RHAU unwinding efficiency towards DNA and RNA substrates. We chose the TERRA telomeric repeat r-GGGUUAGGGUUAGGGUUAGGG as a RNA G4 conformation and the human telomeric sequence h-telo d-GGGTTAGGGTTAGGGTTAGGG, as its G4 DNA counterpart. To assess the ability of RHAU to unwind more efficiently a given substrate based on its DNA or RNA nature, all the systems tested differed only in their central part: their DNA 3' overhang and 5' labelled duplex were kept identical.

First, our results showed that the duplex control, regardless of its DNA or RNA nature, is less efficiently unwound than the mutated (variable central part containing a single-strand) or the G4 systems: only 20% of the systems were unwound by RHAU in our conditions (**Figure 5**). As our results showed that using the mutated systems RHAU can unwind very efficiently a labelled 15 bp duplex (**Figures 1 and 2**), we decided to decrease the length of the central duplex from 21 to 15 bp. Indeed, we wanted to elucidate why RHAU was unable to unfold this additional duplex and one hypothesis could be its length which reflect its thermal stability. Our results demonstrated that shortening the duplex improves RHAU unwinding activity: 60% of the systems (over 20% for the the 21 bp duplex) were unwound in solution (supplementary **Figure S2**). As shortening a duplex decreases its thermal stability, this suggests that, duplex unwinding by RHAU is dependent on its thermal stability.

Second, **Figure 5** also showed that the amount of G4 DNA systems unwound by RHAU is slightly lower than its RNA counterpart TERRA (57±10 % *vs* 73±5 %). Third, as previously observed with the c-myc system, h-telo is less efficiently unwound than its mutated version. In contrast, no significant difference in RHAU unwinding activity is observed between the TERRA and the mutated RNA system. Our results suggest that even if RHAU is able to unwind both substrates, in our present conditions it prefer the RNA substrate than its DNA

counterparts, even if in the case of the G4 bearing sequences the thermal stability of the G4 RNA is higher.

RHAU activity in the presence of Phen DC3 and NMM

G4 ligands were developed to interact with G4-forming sequences in order to interfere with their potential biological roles. They can impede for instance their processing by enzymes such as telomerase or helicases. As our experiments showed that G4 processing by RHAU was not impeded by increasing potassium concentrations, we wanted then to check if this effect was confirmed in the presence of well-known G4 stabilizers. To this aim, we chose to use two G4 ligands: Phen DC3 and NMM. Unwinding of all the G4 systems decreased dramatically in the presence of Phen DC3 (**Figure 6**) while the mutated system was slightly impacted (84±4% *vs* 65±7%) (Supplementary **Figure S4**). In contrast, NMM was unable to inhibit RHAU processing of the G-quadruplex systems (supplementary **Figure S3**).

4. Discussion

G-quadruplex prone sequences are localized in key genomic regions such as oncogene promoters and telomeres.[1] Numerous studies demonstrated their interactions with proteins involved in important biological processes affecting nucleic acid metabolism.[9] In particular, helicases such as BLM, WRN, FANC J or PIF1, which are associated with mutations responsible for cancer predisposition, can interact with G-quadruplexes, highlighting the importance of G4 processing for the basal function of cells.[33,34] As helicases such as WRN and RecQ1 are overexpressed in some cancers, they may constitute interesting therapeutic targets.[35,36]

Telomerase is overexpressed in most human cancers; its activity can be inhibited either by directly targeting the protein itself or indirectly by sequestrating its substrate.[37] Indeed, telomere extension by telomerase was prevented when its substrate adopts a G4 structure and this phenomenon is strengthened by G4 ligands.[38–43] Furthermore, the use of small molecules to enhance G4 stability and impede oncogene expression at the promoter level may constitute another interesting anti-cancer strategy.[44]

Co-immunoprecipitation experiments have shown that RHAU interacts with hTR, the RNA template of telomerase, which may adopt a G4 conformation. It was postulated that RHAU unwinds this G4 to enable telomerase activity.[27] Additionally, it has been shown that RHAU is overexpressed in breast cancer cell lines and interacts with transcription factors playing positive role in cell proliferation and cancers.[17,45] All of these are in favour of a pro-oncogenic role of RHAU both at the telomerase and the promoter levels.

Here, we characterized the interactions of full-length RHAU with G-quadruplex forming sequences. To this aim, we used our previously developed high-throughput fluorescence G4-based assay to follow, in real time, nucleic acid substrates/RHAU interactions under different conditions.[31] We demonstrated that an increase in potassium concentration (up to 100 mM) strengthens G4 stability but does not inhibit significantly RHAU unwinding activity towards the c-myc system. This result is in contrast with our previous observations on ScPif1.[31]

These results suggest that RHAU unwinding activity towards G4 DNA is independent of its thermal stability, which is in contradiction with the findings of Chen and co-workers.[46] They demonstrated that an increase in the thermal stability of a tetramolecular G4 DNA (resulting from a larger tetrad number) impedes RHAU unwinding activity.[46] These different observations could be explained by a preference for a different substrate molecularity

(unimolecular *vs* tetramolecular) and/or by a different way to process them, as shown for BLM. This enzyme is a 3' to 5' helicase, which prefers intermolecular G4 but not intramolecular G4 as compared to duplexes.[47,48] This highlights differences in helicase processing of intermolecular and intramolecular G4 substrates.

In relation to RHAU substrate specificity, in our conditions RHAU unwinds an unfolded control mutated system more easily than the c-myc system even when the protein concentration increases. In contrast, the G4 RNA TERRA sequence is unwound in the same way than its RNA mutated control sequence. This could mean that RHAU processes G4 RNA substrates more easily than the G4 DNA substrates or this could be due to the RNA nature of the substrate. This is hardly surprising as RHAU possesses an RNA binding stretch which should help the RSM process G4 RNA. These results are consistent with previous studies which showed that RHAU affinity towards intramolecular or tetramolecular G4 RNA was higher compared to their DNA counterparts *in vitro*.[15,28]

Regarding duplex processing, we revealed that RHAU failed to unwind 21bp duplexes, whatever their DNA or RNA nature. Nevertheless, decreasing the length of the duplex substrate from 21 to 15 bp restored RHAU unwinding ability. These findings suggest that RHAU unwinding activity towards duplexes as well as tetramolecular G-quadruplex structures may be dependent on their thermal stability, as decreasing the duplex length or the G4 tetrad number lead to a decrease in thermal stability.[46] Those observations suggest that RHAU processing of intermolecular substrates (G4 or duplexes) may involve a mechanism which could be different for unimolecular G4.

Finally, we also assessed RHAU unwinding activity in the presence of G4 ligands. The chosen compounds, Phen DC3 and NMM, belong to the bisquinolinium and porphyrin chemical families.[49,50] These compounds bear different charges (+2 for Phen DC3, -2 for NMM) Despite these differences, both ligands are good G4 stabilizers and share the same interaction mode with G-quadruplex structures, mostly through π-π stacking on the terminal quartet of the G4 structures.[51,52]

Compounds that stabilize G-quadruplexes *in vitro* have been shown to impair helicase activities towards G4 substrates. Indeed, gel-based assays have shown that NMM can inhibit BLM, as well as the yeast helicase Sgs1 unwinding activities towards tetramolecular G-quadruplexes.[53] Phen DC3 was shown to interfere with both yeast and human helicase activities of ScPif1 and RHAU. Gel-based assays demonstrated that Phen DC3 inhibited the helicase activity of RHAU towards a tetramolecular G4 substrate and the one of ScPif1

towards an intramolecular G4 forming sequence.[46,54] We confirmed these results in our conditions using the fluorescent G4-based helicase assay for both ScPif1[31] and RHAU towards intramolecular G4 forming substrates. In contrary, in our conditions, NMM did not inhibit RHAU unwinding activity towards an intramolecular G4 substrate.

The commonly accepted dogma is that G4 ligand inhibition of helicase activity occurs through enhancing substrate stability, but this could be only a part of the explanation. Indeed, it was demonstrated that RHAU N-terminal domain recognizes the terminal tetrad of an intramolecular quadruplex.[15,16] One hypothesis could be the difference in ligand affinities for G-quadruplex-forming sequences. Contrary to Phen DC3, NMM displays relatively weak binding affinity for G4,[50,55] suggesting that the lack of RHAU inhibition of NMM is due to G4 tetrad accessibility. Another hypothesis is that Phen DC3 is a RHAU inhibitor whereas NMM is not. Indeed, Phen DC3 also affects RHAU activity on the mutated system while this is not the case for NMM (**Supplementary figure S4**). This latter hypothesis emphasizes the importance of screening ligand libraries with different helicases to find specific inhibitors, which is easy with our high-throughput assay.

5. Conclusions

RHAU/G4 interactions and more generally helicase/G4 interactions represent an attractive research field to find new anti-proliferative agents. Indeed the fluorescent G4-based assay we developed enables from one hand, a better understanding of the conditions for this interaction and from another hand, the screening of suitable ways to promote or inhibit such reaction. This assay allows a better characterisation of helicase substrate specificity, which cannot be extensively studied by other techniques.

Acknowledgments

We want to thank Lionel Beaurepaire for helpful discussions. This work was supported by a grant from *Agence Nationale de la Recherche* (‘Quardpdiem’ [ANR-12-BSV8-0008-01]) and Conseil Régional d’Aquitaine to J.L.M.

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Figures

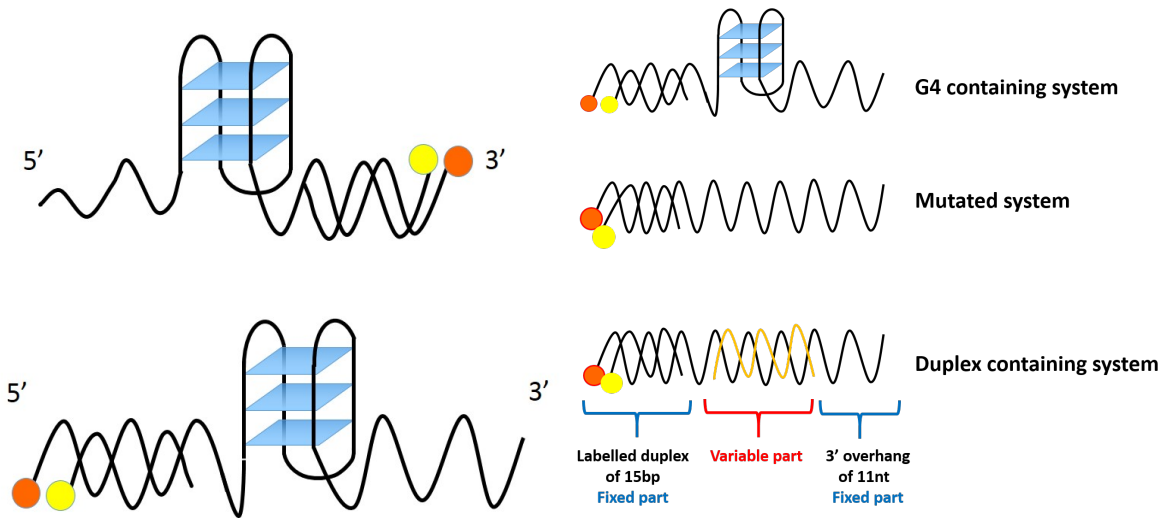

Figure 1: Representation of the nucleic-acid systems.

On the left: G4-containing nucleic acid systems with either a 5' (top) or 3' overhang (bottom). Each system is composed of two strands: a long Dabcyl-labeled strand (bearing the G4 sequence) and a short FAM-labelled strand of 15 nucleotides, which forms a 15-bp duplex when annealed to the Dabcyl-labelled strand.

On the right: 3' overhang nucleic acid systems with two DNA fixed part: a 3' single-strand and a 5' doubly labelled DNA duplex with FAM and Dabcyl. The central part is variable; it can be a G4 forming sequence (top), a simple single-strand (middle) or a duplex (bottom). This part can either be DNA or RNA. For duplexes we also used a DNA:RNA hybrid construction.

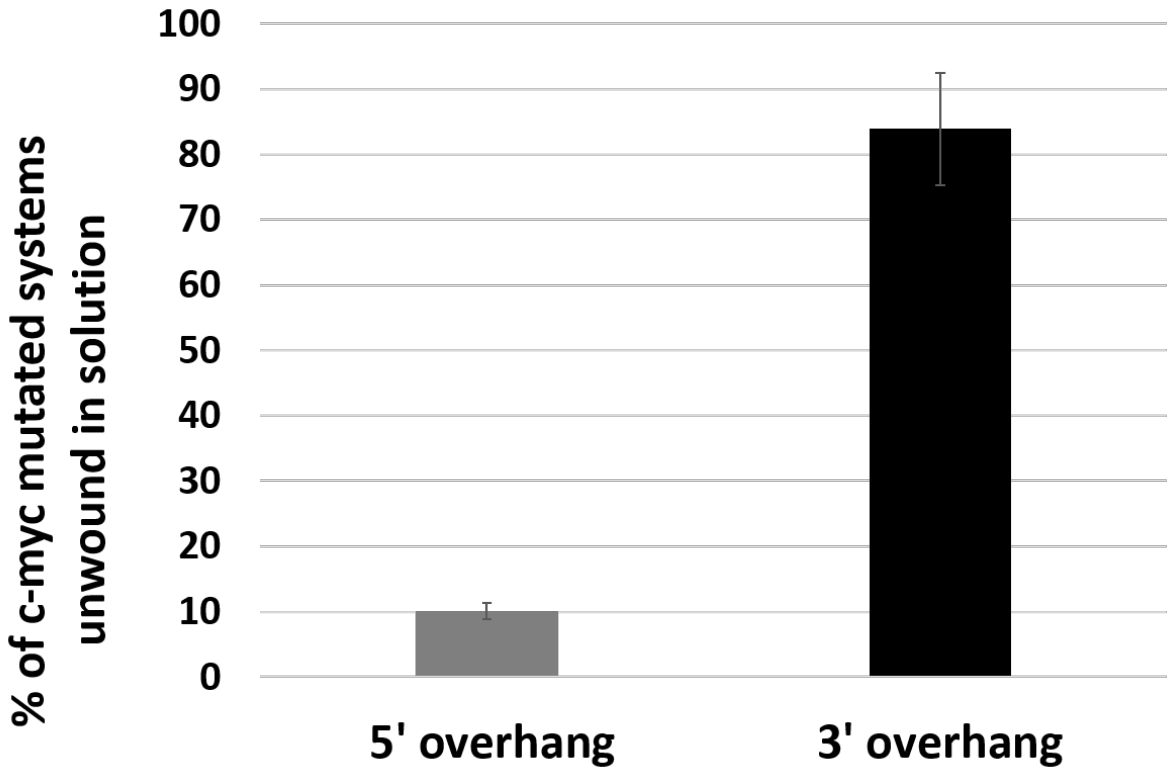

Figure 2: RHAU unwinding activity towards DNA mutated c-myc system, bearing either a 3' or a 5' overhang.

RHAU unwinding activity was performed using 10 nM of DNA nucleic acid system and 5 nM of RHAU helicase in a reaction buffer containing 20 mM Tris-HCl, 5 mM $MgCl_2$ supplemented with 1 mM KCl and 99 mM NaCl. Error bars indicate standard deviation between at least three independent experiments.

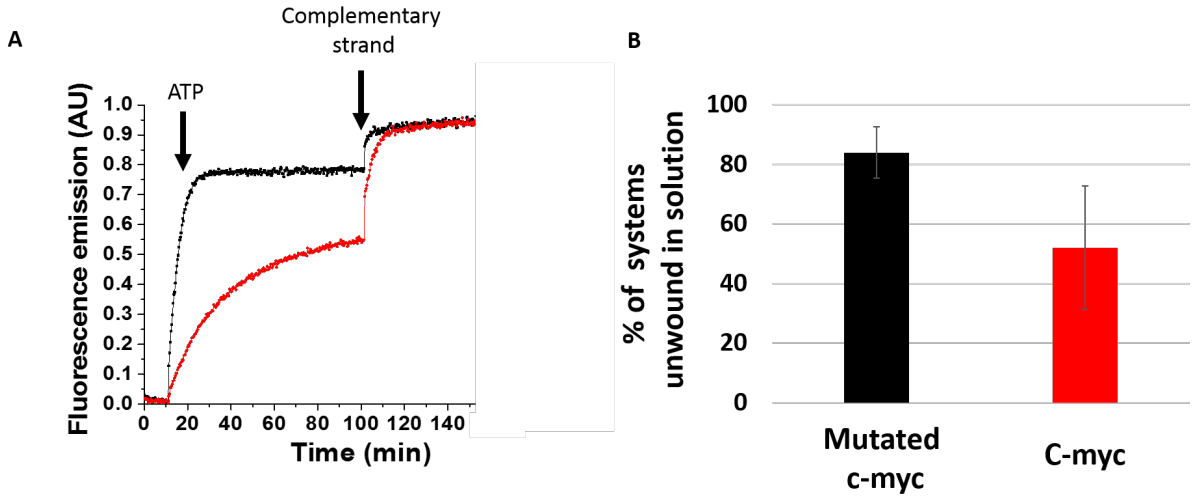

Figure 3: RHAU unwinding activity towards 3' overhang the c-myc system (red) and its mutated control sequence (black).

RHAU unwinding activity was performed using 10 nM of DNA nucleic acid system and 5 nM of RHAU helicase in a reaction buffer containing 20 mM Tris-HCl, 5 mM $MgCl_2$ supplemented with 1 mM KCl and 99 mM NaCl. Error bars indicate standard deviation between at least three independent experiments. (A) Normalized fluorescence emission of systems unwound in solution in function of time. (B) Percentage of system unwound by RHAU in our experimental conditions.

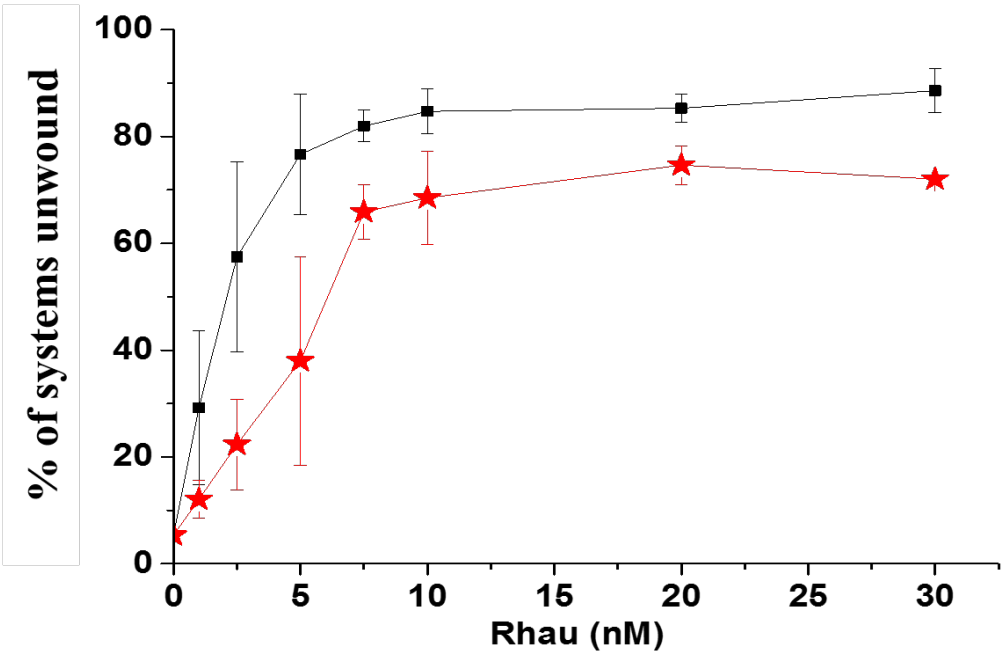

Figure 4: c-myc and mutated DNA systems unwinding as a function of RHAU concentration.

RHAU unwinding activity was followed using 10 nM of DNA nucleic acid system and 1, 2.5, 5, 7.5, 10, 20 and 30 nM of RHAU helicase in a reaction buffer containing 20 mM Tris-HCl, 5 mM $MgCl_2$ supplemented with 50 mM KCl and 50 mM NaCl. Error bars indicate standard deviation between at least three independent experiments. Black squares: percentage of mutated c-myc unwinding; red stars: percentage of c-myc unwinding.

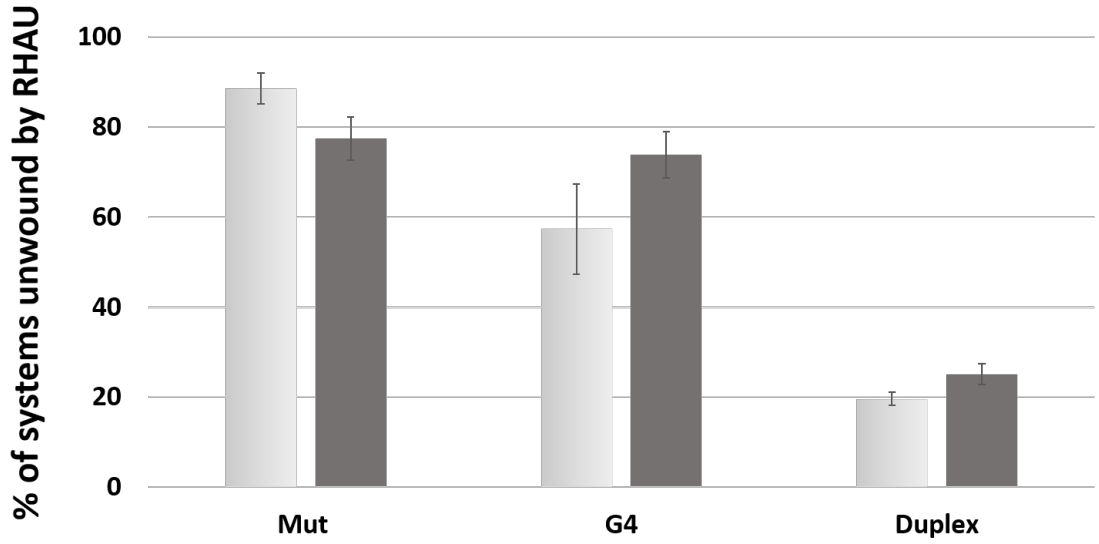

Figure 5: RHAU unwinding activity towards different nucleic acid systems.

RHAU unwinding activity was followed using 10 nM of nucleic acid system and the same concentration of RHAU helicase in a reaction buffer containing 20 mM Tris-HCl, 5 mM $MgCl_2$ supplemented with 50 mM KCl and 50 mM NaCl. Error bars indicate standard deviation between at least three independent experiments. RHAU unwinding activities towards DNA (light grey) and RNA (dark grey) systems, derived respectively from h-telo and its RNA counterpart TERRA, and towards their mutated unfolded controls.

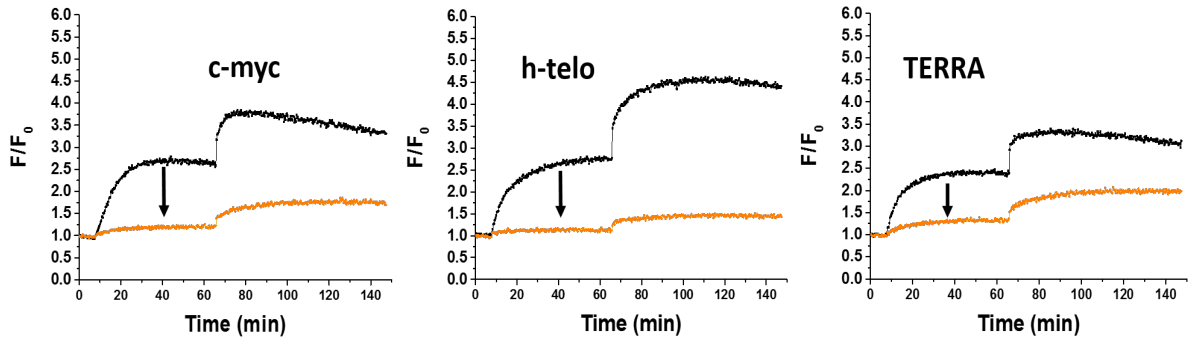

Figure 6: G-quadruplex systems unfolding by RHAU in presence of Phen DC3

RHAU unwinding activities as a function of time were analysed using 10 nM of DNA nucleic acid system and the same concentration of RHAU helicase without G4 ligand (black curves) and in the presence of 12 equivalents of Phen DC3 (orange curves).

Table I: List of the nucleic acid sequences used in this study

Sequence Name	Sequence (5'-3')
Mut c-myc	DABCYL-GAGACGAGTTGCCTTAT **T**GGTG**T**GTAG**T**GTGG**T**A_{11}
C-myc	DABCYL-GAGACGAGTTGCCTTAT **GGG**T**GGG**TA**GGG**T**GGG** A_{11}
H-telo	DABCYL-GAGACGAGTTGCCTTAT **GGG**TTA**GGG**TTA**GGG**TTA**GGG** A_{11}
Terra	DABCYL-GAGACGAGTTGCCTTAT **GGG**UUA**GGG**UUA**GGG**UUA**GGG** A_{11}
Mut H-telo	DABCYL-GAGACGAGTTGCCTTAT **T**GGTTA**GT**GTTA**GGT**TTA**GTG** A_{11}
Mut TERRA	DABCYL-GAGACGAGTTGCCTTAT **U**GGUUA**GU**GUUA**GGU**UUA**GUG** A_{11}
Comp 21bp DNA strand	CACTAAACCTAACACTAACCA
Comp 15 bp DNA duplex	CACTAAACCTAACAC
Comp 21bp RNA strand	CACUAAACCUAACACUAACCA
Comp Mut	T_{11}ACC ACA CTA CAC ACC AAT AAG GCA ACT CGT CTC
Comp c-myc	T_{11}CCC ACC CTA CCC ACC CAT AAG GCA ACT CGT CTC
Comp H-telo	T_{11}CAC TAA ACC TAA CAC TAA CCA ATA AGG CAA CTC GTC TC
Comp TERRA	T_{11}CAC TAA ACC TAA CAC TAA CCA ATA AGG CAA CTC GTC TC
Trap	TTC CGT TGA GCA GAG
FAM-strand	FAM-CTCTGCTCAACGGAA
Mut 5'	5'-$(A)_{11}$-TGGTGTGTAGTGTGGTTTATTCCGTTGAGCAGAG-3'-Dabcyl
Comp-Mut 5'	CTC TGC TCA ACG GAA TAA ACC ACA CTA CAC ACC A T_{11}
Trap 5'	GAG ACG AGT TGC CTT
FAM-strand 5'	AAGGCAACTCGTCTC-5'-FAM