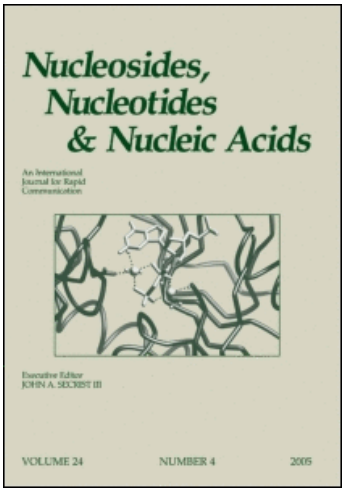

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Formation of Triple Helices at Irregular Poly (R·Y) Sites Located in Critical Positions in the Human *BCR* Promoter

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FORMATION OF TRIPLE HELICES AT IRREGULAR POLY (R•Y) SITES LOCATED IN CRITICAL POSITIONS IN THE HUMAN *BCR* PROMOTER

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ABSTRACT: Two polypurine sequences interrupted respectively by one and two adjacent pyrimidines have been identified in the promoter of the human *bcr* gene. Although these targets are irregular they are recognised and tightly bound by AG and GT motif triplex-forming oligonucleotides. Thermodynamic and kinetic data are presented.

Anti-gene strategies based on the use of triple-forming oligonucleotides (TFOs) as artificial transcription repressors are constrained by the need of genomic targets with a polypurine-polypyrimidine motif [poly (R·Y)]. These sequences occur frequently in 5'-flanking regions of eukaryotic genes, but are often irregular as their polypurine motif is interrupted by one or more pyrimidines[1]. From the biochemical and pharmacological perspective, the anti-gene strategy would have maximum utility if the TFOs could bind to a wide range of potential targets, including the irregular poly (R·Y) sequences located in critical DNA regions for controlling gene expression. However, little is known about the formation of triple helices at irregular targets and the few studies reported in the literature have been conducted on biologically not relevant DNA sequences[2,3]. Recently we focused our attention on two irregular poly (R·Y) sequences located in the promoter of the human *bcr* gene (**FIG**.1). One of these sequences (target 1) spans over transcription initiation and contains two CG inversions, whereas the other (target 2) is located upstream from the TATA box and contains one CG inversion. These targets are ideal for trying to inhibit or down-regulate the expression of the *bcr/abl* fuse gene involved in the genesis of CML leukaemia[4]. To this purpose, we designed AG and GT motif TFOs and studied their ability

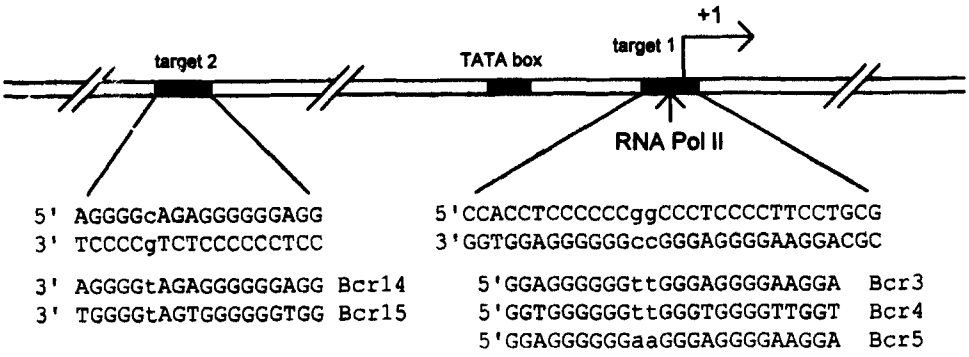

FIG.1: Sequences of the irregular poly (R·Y) targets located in the human bcr promoter and of the designed triple-forming oligonucleotides.

to bind to *bcr* irregular poly (R.Y) sites. The designed TFOs should bind in an antiparallel manner to the major-groove of the targets and juxtapose either C or A to the CG inversions, thus incorporating T·C·G and A·C·G mismatched triads in the triplexes (**FIG.1**).

Electrophoresis mobility-shift experiments (EMSA) showed that the TFOs are able to tightly bind to target 1 and target 2 at 37°C, in 50 mM Tris-acetate, pH 7.4, 10 mM $MgCl_2$, 5 mM NaCl (standard buffer). Typical EMSA are shown in **FIG.2** (top). Dimethyl sulphate and DNase I footprintings showed that the TFOs recognise and bind to the irregular targets in the correct way, without giving rise to detectable amounts of shifted complexes[5]. From EMSA we determined the dissociation equilibrium constants of the triplexes, which were found to vary from 10^{-7} to 10^{-8} M[5] at 37°C (**TAB.1**). These K_D values are comparable to those obtained for the AG and GT motif TFOs designed for the regular poly (R·Y) site contained in the Ki-*ras* promoter[7]. It can be seen that the AG motif TFO Bcr3, which juxtaposes T to the adjacent CG inversions of target 1, forms the most stable triplex. This is probably due to the fact that a water molecule is coordinated between T(N3-H) and G(O6) of T·C·G, so that this triad forms two H-bonds with the purine of the target[6,8], whereas A·C·G does not (**FIG.2**, bottom).

In order to estimate the stability of *bcr* triplexes we performed EMSA and UV/CD melting experiments. We found that the triplexes formed at the target 1 and 2 migrated in a polyacrylamide gel thermostated at 50°C without showing any dissociation, suggesting

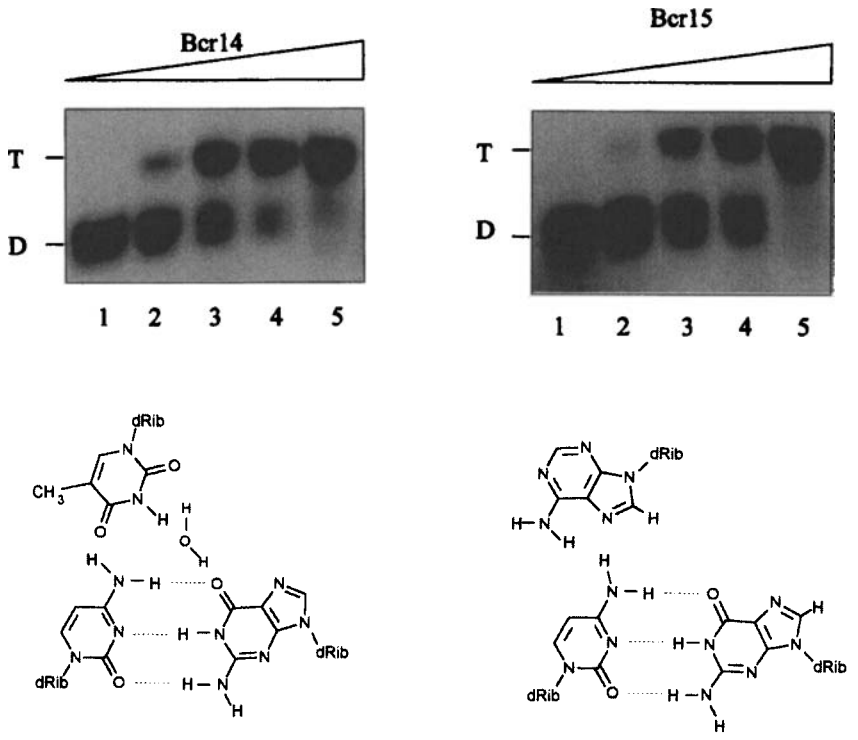

FIG.2 (Top) EMSA relative to the reaction of triplex formation between oligonucleotides Bcr14, Bcr15 and target 2, in 50 mM Tris-acetate, pH 7.4, 5 mM NaCl, 10 mM $MgCl_2$. Lane 1 contains target 2 (28 nM), whereas lanes 2 to 5 have been loaded with target 2+TFO mixtures at ratio 1:1, 1:5, 1:10 and 1:100, respectively; (Bottom) Probable structures of T·C·G and A·C·G mismatched base triplets.

TABLE 1

Termodynamic parameters for the interaction between the desigend TFOs and the irregular *bcr* poly (R·Y) sites (target 1 and target 2 of FIG.1)

TFO (M)	K_D * (kcal/mol)	$\Delta G_{(37)}$
Bcr3	6.4×10^{-8}	10.2
Bcr4	3.1×10^{-7}	9.3
Bcr5	3.5×10^{-7}	9.7
Bcr14	1.6×10^{-7}	9.6
Bcr15	2.7×10^{-7}	9.3

*uncertainty of the data is about ±10%

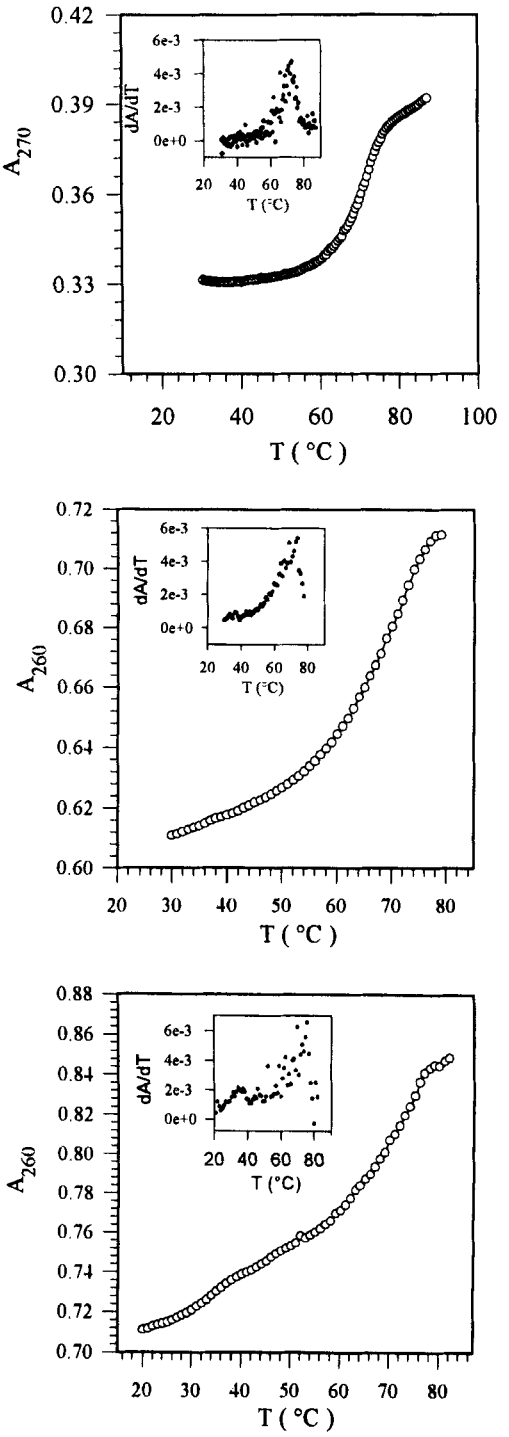

FIG.3 (Top) Melting curve of target 2 in 50 mM Tris-acetate, pH 7.4, 50 mM NaCl, 10 mM $MgCl_2$, which gives a Tm of 68°C; (Middle) melting curve of the triplex formed by Bcr14 and target 2, giving a Tm of 72°C; (Bottom) melting curve of the triplex formed by Bcr15 and target 2, giving a Tm of 72°C.

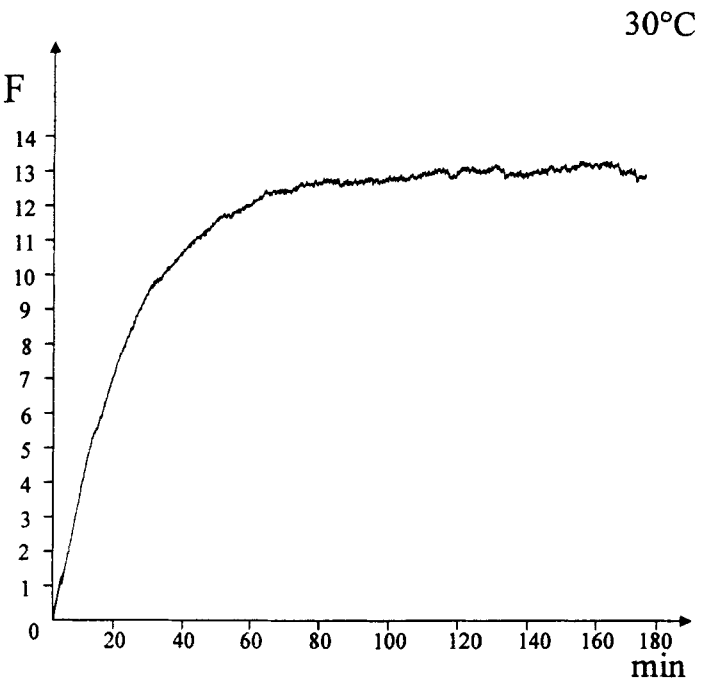

FIG.4 Increase of fluorescence as a function of time, following the addition of Bcr3-fluorescein (0.1 μM) to target 1 of *bcr* (0.7 μM) at 30°C in 50 mM Tris-acetate, pH 7.4, 50 mM NaCl, 10 mM $MgCl_2$, 2 mM spermidine. The ordinate, F, is expressed in arbitrary units.

that these triplexes have Tm higher that 50°C. The triplex formed at target 2 was also examined by UV-melting experiments in standard buffer. **FIG**.3 shows that while the target melts at 68°C, the triplexes made by Bcr14 and Bcr15 are found to melt at 72°C. Similar results were obtained with circular dichroism (not shown). The lower temperature transition present in the melting curve of the triplex formed by Bcr15 is due to the disruption of self-associated structures formed by the G-rich TFOs.

We also wished to investigate the rate at which the triplexes are formed at the *bcr* irregular poly (R·Y) sites. To this purpose Bcr3, labelled at the 3'-end with fluorescein, was mixed with an excess of target 1 and the increase of fluorescence (λ excit.= 480 nm, λ emiss.= 521 nm) accompanying the reaction of triplex formation was recorded as a function of time. Plotting the fluorescence increase against time we obtained exponential curves as shown in **FIG**.4, which showed that the TFO binds to the target within 15 min.

Finally, we observed that these triplexes are not strongly destabilised by physiological K^+ and Na^+ concentrations and are instead stabilised by polyamines[9]. To conclude, the binding properties of the designed TFOs have encouraged us to test them *in vivo* on 293 Ph+ leukaemia cells, in order to attempt an artificial inhibition of the fuse *bcr/abl* gene.

REFERENCES

1. Behe M.J. *Nucleic Acids Res.*, **1995**, *23*, 689-695.
2. Gowers, D.M.; Fox, K.R. *Nucleic Acids Res.*, **1997**, *25*, 3787-3794.
3. Kukreti, S.; Sun, J.; Garestier,T.; Hélène, C., *Nucleic Acids Res.*, **1997**, *25*, 4264-4270.
4. Shtivelman, E.; Lifshitz, B.; Gale, R.; Canaani, E. *Nature*, **1985**, *315*, 550-554.
5. Xodo, L.; Manzini, G.; Quadrifoglio, F. *Ant.Nucl.Ac.Drug Dev.*, **1988**, in press.
6. Dittrich,K.; Gu, J.; Tinder, R.; Hogan, M.; Gao,X. *Biochemistry*, **1994**, *33*, 4111-4120.
7. Alunni M.; Pirulli, D.; Manzini, G.; Xodo, L. *Biochemistry*, **1996**, *25*, 16261-16369.
8. Weerasinghe, S.; Smith, P.; Pettitt, M.*Biochemistry*, **1995**, *34*, 16270-16278.
9. Xodo, L.; Flores, R.; Manzini, G.; Scaggiante, B.; Quadrifoglio, F., submitted.