Vol. 116, No. 1, 1983 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
October 14, 1983 Pages 113-120

DETECTION OF SEQUENCES WITH Z-DNA FORMING POTENTIAL IN HIGHER PLANTS

Michel Delseny, Monique Laroche and Paul Penon

Laboratoire de Physiologie Végétale - ERA N°226
Université de Perpignan - 66025 Perpignan (France)

Received August 26, 1983

Sequences of alternating purine-pyrimidine residues with Z-DNA forming potential have been detected in the nuclear DNA of two higher plant species : wheat and radish. Poly (dG-dT) and poly (dG-dC) stretches have been detected by hybridization of the corresponding nick-translated probes to Southern blots. These stretches are scattered throughout the genome and some of them belong to moderately repeated sequence families interspersed with other DNA sequences.

DNA sequences containing alternating purine and pyrimidine residues can exist in a left-handed conformation of the double helix or in the more common right-handed B - form (1). Transition from right-handed B form to left-handed Z-form is energetically favoured in negatively supercoiled plasmids (2, 3). It can be induced by concentrated salts (4) or in the presence of Mn^{++} and a brief exposure to elevated temperature (5). The left handed structure can also be stabilized at physiological ionic strength by cytosine methylation (6). Antibodies which specifically recognize Z-DNA have been raised and used to demonstrate that this configuration occurs in natural DNA sequences (7, 8). Another approach consists in using alternating purine-pyrimidine synthetic polynucleotides to detect sequences with Z-DNA forming potential in Southern blot hybridizations. Stretches of poly (dG-dT) and poly (dG-dC) have thus been shown to be widely spread in several eukaryotic genomes (9). So far these sequences have not been looked for in the nuclear genome of higher plants. The biological function of left-handed DNA is not yet clear. Transition from B to Z form induces changes in supercoiled plasmids by modifying the superhelicity. Such changes are likely to occur in chromatin where they might play a role in controling gene expression. The suggestion that Z-DNA plays a role in regulating gene expression is reenforced by the isolation, from Drosophila nuclei, of proteins which specifically bind to Z-DNA (10) and by the recent finding that the negatively supercoiled form of simian virus

0006-291X/83 $1.50

SV 40 DNA contains Z-DNA segments within the transcriptional enhancer sequences (11). Several other viral genomes display similar Z-DNA stretches in enhancer regions involved in the control of transcription (11). Z-DNA has been used as a template for *Escherichia coli* RNA-polymerase (12) and studies have been initiated to investigate the effect of a B to Z transition on the activity of wheat germ RNA-polymerase II (13). In the absence of any published evidence for potential Z-DNA in higher plants, it seemed worth-while to investigate this point.

In this report we present hybridization data which demonstrate that oligo (dG-dT) and oligo (dG-dC) tracts occur in the nuclear genome of wheat and radish.

MATERIALS AND METHODS

DNA from wheat and radish was prepared from a crude nuclear pellet by sarkosyl lysis in the presence of ethidium bromide. The lysate was adjusted with CsCl to a refractive index of 1.39 and centrifuged for 3 days at 30000 rpm at 20°C (14). The DNA band was collected and the ethidium bromide removed by four extractions in the presence of isoamyl-alcohol. This DNA was then mixed with CsCl and the density adjusted to 1.696 $g.cm^{-3}$ for radish and 1.700 $g.cm^{-3}$ for wheat. These solutions were centrifuged to equilibrium for 3 days at 30000 rpm. The gradients were eluted and the peak fractions were pooled, dialysed and precipitated in the presence of ethanol. DNA was digested with restriction enzymes according to suppliers instructions. Eco RI and Alu I were from Boehringer, Hae III was prepared and gifted to us by Dr Gérard ROIZES (University of Montpellier). Digested DNA was run on agarose gels and blot transferred to nitrocellulose sheets as already described (15-16). Poly (dG-dT) and poly (dG-dC) were from P-L Biochemicals and were kindly given to us by Dr Dominique JOB (CNRS -MARSEILLE). These polynucleotides were labelled by nick translation according to published procedures (17) using ^{32}P α dATP (400 ci/mM) for poly (dG-dT)and ^{32}P α dCTP (400 ci/mM) for poly (dG-dC). Both tracers were from Amersham Ltd (UK). Hybridizations were carried out overnight at 60°C in sealed plastic bags in the presence of 4xSSC (SSC is 0.15 M NaCl 0.015 M sodium citrate) ,0.08% serum albumin, 0.08 % ficoll, 0.08% polyvinylpyrolidon and 0.1% SDS. Filters were washed at 65°C with 1 liter 3xSSC, 0.1% SDS (three 30 minute washes with 300-350 ml per filter)and then with 1 liter 0.3 x SSC (three 30 minute washes with 300-350 ml per filter). Two recombinant bacteriophage DNAs and one plasmid have been used in this work. Phages λ RA 2 and λ RA 38 contain Eco RI fragments from radish rDNA. λ RA 2 contains two Eco RI fragments spanning the large external spacer and most of the 18 S rRNA coding sequence. λ RA 38 contains three Eco RI fragments spanning 18S rRNA, transcribed spacer and most of the 25 S rRNA coding sequences. The two phages have been isolated from a partial library constructed by inserting partial Eco RI digests into phage λ gt WES/λ B (our unpublished results). Plasmid pRB 12 contains a radish satellite DNA insert made of eight ≈ 180 bp repeat units. It was isolated from a plasmid library prepared by inserting Hind III fragments of rDNA rich-DNA from radish at the Hind III site of plasmid vector pAT 153 (our unpublished results).

RESULTS

1) Detection of poly (dG-dT) tracts.

Wheat DNA was digested with three different restriction enzymes : Eco RI, Alu I and Hae III. The two latter enzymes recognize a four base pair sequence and cut much more frequently than Eco RI. The resulting fragments were analysed on a 1% agarose gel and probed with ^{32}P poly (dG-dT)·poly (dC-dA). Figure 1 shows the result of this experiment. An ethidium-bromide stained gel picture is shown for comparison. The Eco RI pattern (lane 3)gives a smear, ranging from more than 25kb to 1.5 kb. Two bands at 3 and 3.7 kb are poorly visible, superimposed to the smear. The Alu 1 (lane 4) and Hae III (lane 5) patterns also give a smear but a series of discrete bands are clearly visible on the autoradiograph. Alu I completely digests the wheat genome whereas a fraction is resistant to Hae III in our experimental conditions. This resistant fraction contains an appreciable proportion of the poly (dG-dT) stretches. Major Alu I bands are at 1450, 1050,940,

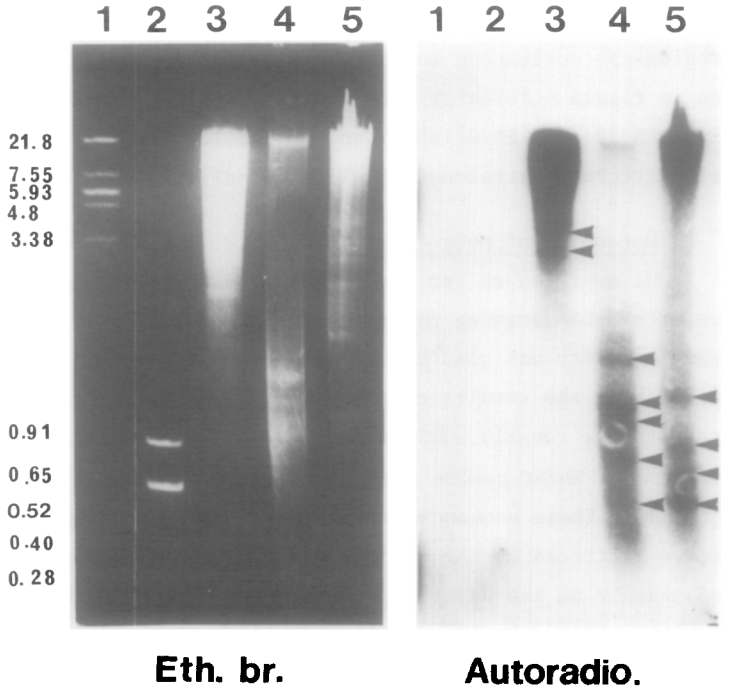

Figure 1.- *Detection of oligo (dG-dT) stretches in wheat nuclear DNA.*

Left:1% agarose gel electrophoresis of wheat DNA digested with Eco RI (3), Alu I (4) and Hae III (5). Lanes 1 and 2 show lambda DNA digested with Eco RI and pBR 322 digested with Alu I as size markers. Sizes are indicated in kbp on the left.Right:autoradiograph of a Southern blot of the same gel probed with ^{32}P poly (dG-dT)-poly (dC-dA). Arrowheads indicate bands superimposed to the background smear.

770 and 530 bp. Hae III major bands are at 1160, 800, 670 and 530 bp respectively. These results indicate that poly (dG-dT) tracts are scattered in the genome and that some of them are associated with specific repeated DNA families of discrete size. Since the sizes of the different families do not constitute a series of multimers, these families are not clustered in tandem repeats but instead are interspersed with other sequences. The Alu I and Hae III families have different sizes and therefore correspond to different sequences.

Figure 2a,b shows that poly (dG-dT) tracts also occur, with a similar organization, in the genome of radish. Some stretches of oligo (dG-dT) belong to repetitive elements interspersed with other sequence as evidenced by the presence of discrete bands superimposed to the background smear. Several of the bands which are visible on the ethidium bromide stained pattern have been identified in previous work (16 and our unpublished results) as rDNA (triangles on the Eco RI pattern) and as satellite DNA (arrows on Alu I pattern). Since some of these fragments have been cloned it was possible to check whether or not the discrete bands on the autoradiograph correspond to these fragments. The results presented in figure 2c,d clearly show that none of the fragments cloned in λ RA 38 or λ RA 2 (rDNA) and in pRB 12 (satellite DNA) contain detectable stretches of oligo (dG-dT).

2) Detection of poly (dG-dC) tracts.

We next looked for the presence of another type of sequence with Z-DNA forming potential : poly (dG-dC)-poly (dC-dG). The same approach was used but the probe was ^{32}P poly (dG-dC). Figure 3 shows the results of the analysis of the wheat genome. The results are roughly similar to those obtained using the poly (dG-dT) probe. Wheat genome contains sequences hybridizing with poly (dG-dC). These sequences are revealed as a smear and are therefore scattered in the genome. Again, discrete bands are detected, mainly on the Hae III pattern. They define two major Hae III families 1000 and 850 bp long containing oligo (dG-dC) stretches. Such sequences are also detected in the radish genome but bands are barely detectable (not shown).

DISCUSSION

In this report we demonstrate the presence of tracts of alternating purine-pyrimidine residues in the genome of two plants, wheat and radish, a monocot and a dicot. These sequences are of

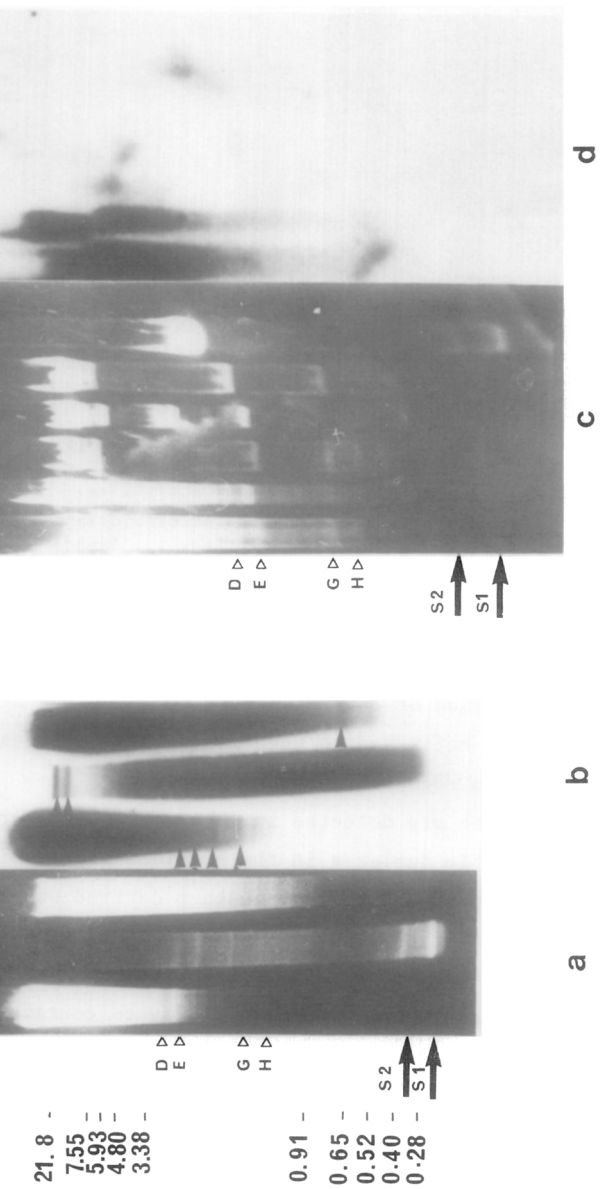

Figure 2. - *Detection of oligo (dG-dT) stretches in radish nuclear DNA.* Left: a and b are respectively an ethidium bromide stained 1% agarose gel and the corresponding Southern blot probed with 32P poly (dG-dT)-poly dC-dA). Lane 1: Eco RI, lane 2: Alu I, lane 3: Hae III. Arrowheads on the autoradiograph indicate discrete bands. Open triangles indicate rDNA Eco RI fragments which have been identified in previous work (16) and which have been cloned in a lambda vector. S1 and S2 arrows point to major Alu I fragments resulting from digestion of the principal radish satellite DNA. S1 is the monomer 180 bp unit, S2 is a dimer. Right: c and d are respectively gel and autoradiograph of the corresponding Southern blot. Lane 4: Eco RI digest of radish DNA, lane 5: Bam HI digest, lane 6: Eco RI digest of DNA of phage λ RA 38(a recombinant containing rDNA Eco RI fragments E G and H), lane 7: Bam HI digest of λ RA 38 DNA, lane 8: Eco RI digest of λ RA 2 DNA (λ RA 2 is a recombinant containing fragments D and G), lane 9: Hind III digest of plasmid pRB 12 showing satellite monomer and dimer unit. Sizes of the standard markers are indicated in kbp.

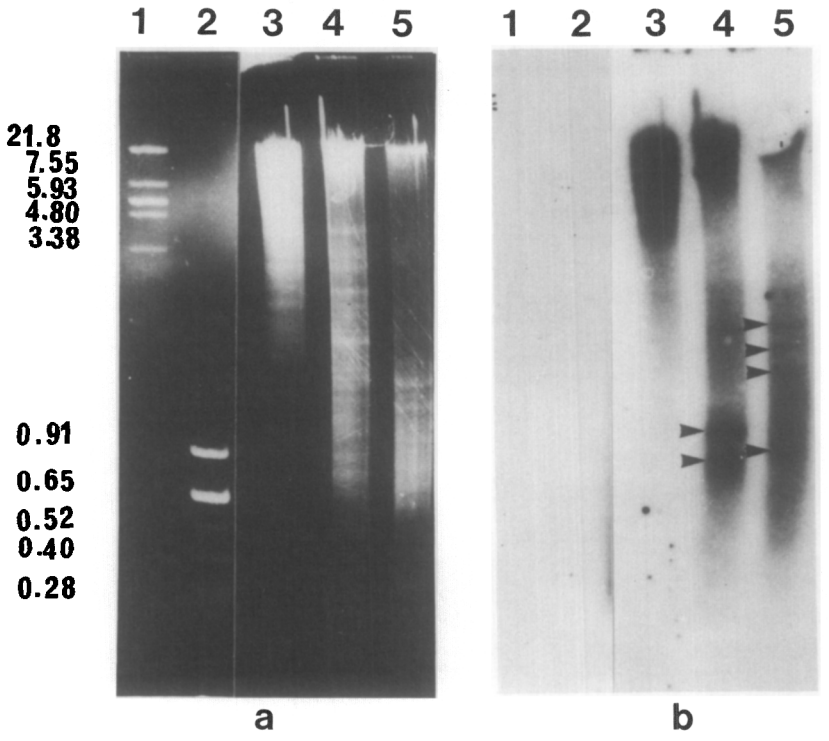

Figure 3.- *Detection of poly (dG-dC) in wheat nuclear DNA.* a and b are ethidium bromide stained gel and autoradiograph of a Southern blot probed with ^{32}P poly (dG-dC). Lane 1 : lambda DNA digested with Eco RI, lane 2 : pBR 322 digested with Alu I, lane 3: wheat DNA Eco RI, lane 4 : wheat DNA digested with Hae III, lane 5: wheat DNA digested with Alu I: Arrowheads point to bands which show up superimposed to the background smear.

particular interest because of their capacity to adopt the Z-conformation of the double helix and to undergo topology changes which may play a role in regulating gene activity.

The sequences which are detected in such an analysis are long enough to form stable duplexes in the hybridization conditions. The hybridization and washings have been carried out in relatively stringent conditions and no spurious hybridization to lambda and pBR 322 size marker fragments was observed. Although it is not certain that all the hybridizing fragments contain a precisely alternating purine-pyrimidine sequence, it is nevertheles likely that most of them contain tracts of alternating sequences 15-20 bp long. Therefore it is clear that these elements which have already been detected in animals and fungi also occur in higher plants. This is confirmed by a survey of some published plant DNA sequences. For instance a clone containing a soybean actin gene (18) and another one containing a maize zein gene (19) have both two tracts of 9-17 alternating purine-pyrimidine resi-

dues a few hundred base pairs upstream the 5' end of the coding sequence. In the absence of any information about the transcription controlling signals in plants we can just speculate that these stretches of potential Z-DNA are part of a regulatory sequence as are similar sequences in some animal viruses (11).

Another interesting point is that some of these stretches belong to interspersed repetitive elements. So far very little is known about these elements in higher plants except that their existence is suggested by DNA denaturation - reassociation kinetic studies (20). Presently it is difficult to determine the copy number of these sequences because they belong to different families. Several highly repeated sequences, tandemly arranged have been isolated by cloning plant DNA but clones corresponding to moderately repeated interspersed elements have not yet been reported. The main difficulty is that no probe is available to fish them out of a genomic library. The use of synthetic polynucleotides such as those used in this study can help to isolate some of these elements and to obtain information concerning their biological function.

ACKNOWLEDGEMENTS

This work was supported by the CNRS (ERA n°226 and ATP Biologie Moleculaire Vegetale). The authors wish to thank Dr D. JOB for stimulating discussions and informations about Z-DNA.

REFERENCES

1. Wang, A.H.J., Quigley, G.J., Kolpak, F. J., Crawford, J.L., Van Boom, J.H., Van der Marel, G. and Rich,A. (1979) Nature, 287, 680-686.
2. Singleton, C.K., Klysik, J., Stirdivant, S.M., and Wells, R.D. (1982) Nature, 299, 312-316.
3. Peck, L.J., Nordheim, A., Rich, A. and Wang, J.C. (1982) Proc. Nat. Acad. Sci. USA, 79, 4560-4564.
4. Pohl, F.M. and Jovin, T.M. (1972) J. Mol. Biol. 67, 375-396.
5. Van de Sande, J.M., Mc Intosh L.P. and Jovin, T.M. (1982) The EMBO J. 1, 777-782.
6. Behe, M. and Felsenfeld, G. (1981) Proc. Nat. Acad Sci USA 78, 1619-1623.
7. Lafer, E.M., Moller, A., Nordheim, A., Stollar, B.D. and Rich, A. (1981) Proc. Nat. Acad. Sci. USA 78, 3546-3550.
8. Nordheim, A., Pardue, M.L., Lafer, E.M., Moller, A., Stollar, B.D. and Rich A. (1981) Nature, 294, 417-422.
9. Hamada, H., Petrino, M.G. and Kakunaga, T. (1982) Proc. Nat. Acad. Sci USA. 79, 6465-6469.
10. Nordheim, A., Tesser, P., Azorin, F., Kwon, Y. H., Moller, A. and Rich A. (1982) Proc. Nat. Acad. Sci USA 79, 7729-7733.
11. Nordheim, A. and Rich, A. (1983) Nature 303, 674-679.
12. Van de Sande, J.H. and Jovin, T.M. (1982). The EMBO J. 1,115-120.

13. Durand, R., Job, C., Zarling, D.A. Teissere, M., Jovin, T.M. and Job D. (1983). Manuscript submitted to The EMBO Journal.
14. Shah, D.M., Nagao, R.T., Eckenrode, V.K. and Meagher, R.B. (1980) Plant Molecular Biology Newsletter 1, 33-34.
15. Delseny, M. and Hull, R. (1983) Plasmid 9, 31-41.
16. Delseny, M., Cooke, R. and Penon, P. (1983) Plant Science Lett. 30, 107-119.
17. Rigby, P.W.J., Dieckmann, M., Rhodes, C., and Berg, P. (1977) J.Mol. Biol. 113, 237-251.
18. Shah, D.M., Hightower, R.C., and Meagher, R.B. (1982) Proc. Nat. acad. Sci. USA 79, 1022-1026.
19. Hu, N.T., Peifer, M., Heidecker, G., Messing, J., and Rubinstein, I. (1982) The EMBO Journal 1, 1337-1312.
20. Walbot, V., and Goldberg, R., (1979) In. Nucleic Acids in Plants T.C. Hall and J.W. Davies, Eds. CRC Press, pp. 3-40.