A long polypyrimidine:polypurine sequence in 5' flanking region of arylsulfatase gene of sea urchin embryo

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ABSTRACT Sea urchin *(Hemicentrotus pulcherrimus)* arylsulfatase(Ars) gene contains a long (622 bp) polypyrimidine:polypurine (Pyr-Pur) sequence in its 5' flanking region. The Pyr-Pur sequence inserted into a plasmid was sensitive to S1 nuclease at a low acidic pH (pH 5) when the plasmid was negatively supercoiled. From the distribution pattern of S1 sites in the Pyr-Pur region it is concluded that a $(CT)_{11}$:$(GA)_{11}$ tract in this region could adopt an unusual DNA configuration distinct from the usual B-form. Another feature of the Pyr-Pur sequence is that this $(CT)_{11}$:$(GA)_{11}$ tract is sandwiched by two oligo(dC):oligo(dG) stretches (G-strings) that are located at almost an equal distance from both ends of the $(CT)_{11}$:$(GA)_{11}$ tract. Mobility shift assay and DNase-I footprinting revealed that the gastrula nuclei contain nuclear proteins that interact with two distinct oligo(dC):oligo(dG) tracts (G-strings) in the Pyr-Pur region. The possibility is suggested that G-strings may be related to formation and stabilization of an unusual DNA configuration of a $(CT)_{11}$:$(GA)_{11}$ tract.

KEY WORDS: *polypyrimidine:polypurine, gene expression, sea urchin, triplex DNA, G-string*

Introduction

In the sea urchin embryo the activity of arylsulfatase (Ars), an enzyme related to the metabolism of sulfated proteoglycans that are believed to play an essential role in cell movement during gastrulation (Akasaka and Terayama, 1983; Akasaka *et al.*, 1990a), begins to increase prior to the onset of gastrulation (Rapraeger and Epel, 1981; Sasaki *et al.*, 1987a), and the amount of the enzyme protein occupies as much as 0.5% of the total protein of pluteus larvae. The increase in the arylsulfatase activity is regulated transcriptionally (Sasaki *et al.*, 1987b), and its transcription begins at the hatching blastula stage, reaching a 2000-fold increase by the prism stage (Sasaki *et al.*, 1988). The Ars mRNA accumulates only in an aboral ectoderm of the gastrula (Akasaka *et al.*, 1990b), suggesting that expression of the Ars gene is regulated spatially as well as temporally at the level of transcription.

Sequence analysis of the Ars 5' flanking region of the sea urchin, *Hemicentrotus pulcherrimus* (Akasaka *et al.*, 1994), and expression assay of Ars-luciferase fusion gene (luciferase assay) (Morokuma *et al.*, in preparation) showed the existence of a long (622 bp) polypyrimidine:polypurine (Pyr-Pur) sequence containing *cis*-acting elements for the Ars gene transcription. A Pyr-Pur sequence is also conserved in the 5' flanking region of the Ars gene of other species of sea urchin, *Strongylocentrotus purpuratus* (Yang *et al.*, 1989). Pyr-Pur regions which have pyrimidines predominantly on one strand have often been reported in the regulatory regions of other eukaryotic genes and are considered to adopt an unusual DNA configuration (Hentschel, 1982; Mace *et al.*, 1983; Htun *et al.*, 1984; Christophe *et al.*, 1985; Finer *et al.*, 1987) like a triplex DNA that is supposed to be related to regulation of transcription (Kohwi-Shigematsu *et al.*, 1983; Emerson and Felsenfeld, 1984).

Several models have so far been proposed for the configuration adopted by the Pyr-Pur region in a supercoiled plasmid based on its S1-nuclease-nicking pattern (McKeon *et al.*, 1984; Evans and Efstratiadis, 1986; Wells, 1988; Wells *et al.*, 1988). A widely accepted model is an intramolecular triplex in which $CGCH^+$ and TAT triads are formed over approximately half of the sequence with about one-half of the complementary purine strand remaining single stranded (Mirkin *et al.*, 1987; Htun and Dahlberg, 1988). Nuclear proteins which interact with Pyr-Pur region have been identified and purified in several organisms (Lewis *et al.*, 1988; Winter and Varshavsky, 1989). In *Drosophila* embryos, protein factors were identified that bind to alternating C and T residues in the promoter regions of histone and HSP genes (Gilmour *et al.*, 1989), and it is suggested that these proteins might participate in

Abbreviations used in this paper: ARS, asylsulfatase; Pyr-Pur, polypyrimidine:polypurine; Pyr-box, pyrimidine box.

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Fig. 1. Nucleotide sequence of the pyrimidine strand of the polypyrimidine:polypurine (Pyr-Pur) region in the 5' flanking region of the sea urchin *(Hemicentrotus pulcherrimus)* Ars gene. *A highly pyrimidine-rich sequence (Pyr-box) is boxed (263 bp, from -1,929 to -1,667) and a* $(CT)_{11}$:$(GA)_{11}$ *tract within this box is underlined.*

either the formation or maintenance of specific DNA structure of promoters, though the functions of these proteins are still unclear.

In the present study, we show that the highly CT-rich 263 bp segment (Pyr-box) in the 622 bp Pyr-Pur region of the sea urchin Ars gene is highly sensitive to an S1 nuclease attack. Based on the distribution pattern of S1 sites we conclude that this box can adopt an unusual DNA configuration, possibly a triplex-DNA. Nuclear proteins interacting with two distinct G-strings in the Pyr-box are also detected.

Results

Some features of polypyrimidine:polypurine region in 5' flanking region of the Ars gene

The Ars gene of the sea urchin, *Hemicentrotus pulcherrimus* contains a polypyrimidine:polypurine (Pyr-Pur) sequence of a 622 nucleotide length from -2,188 to -1,667) in its 5' flanking region (Fig. 1). This sequence contains pyrimidines predominantly in a sense strand and purines in the complementary strand. The 3' half of this Pyr-Pur region is especially enriched in pyrimidines with 96% of nucleotides of this region consisting of C and T residues. This 263 bp region spanning from -1,929 to -1,667 is hereafter called a Pyr-box. This box is featured by the presence of one $(CT)_{11}$:$(GA)_{11}$ tract (from -1,793 to -1,772), several oligo(dC):oligo(dG) tracts (G-strings) and some oligo(dT):oligo(dA) tracts.

S1-nuclease sensitivity of the Ars Pyr-Pur region

An S1 nuclease assay has often been used to detect single stranded DNA regions or other altered conformations, such as a cruciform looping, a B-Z junction and a triplex configuration (Panayotatos and Wells, 1981; Singleton *et al.*, 1982; Fowler and Skinner, 1986), as S1 nuclease specifically attacks the sites of conformational perturbations in duplex phosphodiester backbones of DNA (Evans and Efstratiadis, 1986; Wells, 1988; Winter and Varshavsky, 1989). Because it is reported that various natural and/or artificial polypyrimidine sequences can take triplex DNA configurations that are sensitive to S1 nuclease under certain conditions (Pulleyblank *et al.*, 1985), S1 nuclease sensitivity of the Pyr-Pur region of the Ars gene was tested under the following experimental scheme.

Four different plasmids each containing a distinct sequence of the *Ars* 5' flanking region (Fig. 2A) were constructed, treated with S1 nuclease at pH 5 as described in Materials and Methods, and electrophoresed in an agarose gel. The presence of a single-stranded region or other conformational perturbation was monitored by the appearance of open circular plasmids that were generated by S1 nuclease-nicking of the *Ars* sequence in supercoiled plasmids. As shown in Fig. 2B, closed circular configurations of pArs24 as well as pArs9, both of which contain the Pyr-Pur region, changed into open circular forms after treatment with S1 nuclease (lane 3 and 7). pArs7 that was constructed by deleting the *Ars* sequence upstream beyond the Pyr-Pur region of the pArs9 was

also sensitive to S1 nuclease (lane 9), while pArs15 that lacks the Pyr-Pur region was insensitive to S1 nuclease (lane 5). These results suggest the existence of the S1 nuclease-sensitive DNA configuration in the Pyr-Pur region, while the AT-rich sequence present around the Pyr-box appears to be unrelated to this specific structure.

Mapping of S1 nuclease-sensitive sites in the Pyr-Pur region

To gain further insight into the DNA configuration adopted by the Pyr-Pur region of the Ars gene, we mapped S1 nuclease-sensitive sites in this region.

pArs7 was treated with S1 nuclease, digested with restriction enzymes, radioactively labeled and electrophoresed in a denaturing gel. A typical result of the S1-nuclease assay was presented in Fig. 3A. Almost all S1 sites were found within the Pyr-box forming several clusters. As shown schematically in Fig. 3B, there were three clusters of highly S1-sensitive sites in the purine strand, a 25nts sequence that includes 5nts of the 5' half of the $(CT)_{11}$:$(GA)_{11}$ tract, a 15nts sequence adjacent to the 3' end of this tract, and a 15nts sequence from -1,662 to -1,648. The first and second clusters were characterized by their enrichment with adenines. In contrast to the purine strand, in the pyrimidine strand only one major cluster of S1 sites was detectable in the T-rich region complementary to the second cluster of S1 sites in the purine strand. No S1 sites were detectable either in upstream or in downstream of the Pyr-box. When linearized, pArs7 completely lost its sensitivity to S1 nuclease (data not shown).

The high S1-nuclease sensitivity of the plasmid strongly suggests that when the plasmid is highly negatively supercoiled, the DNA configuration of the Pyr-box in the plasmid shifts from a normal B-configuration to the unusual one that contains strand separation.

Proteins that bind to the Pyr-Pur region

Proteins that interact with the Pyr-box of the Ars gene were screened from nuclear extracts of gastrulae by mobility shift assay. As shown in Fig. 4, several protein-DNA complexes were detected using the two probes, Probe I (286p from -1,880 to -1,595 bp) and Probe II (274 bp from -2,109 to 1,836 bp). Sequence-specificity of the protein-DNA interaction was confirmed by competition experiments. While addition of the plasmid DNA had little effect on the formation of protein-DNA complexes (lanes 5, 6, and lanes 11, 12), the addition of an excess amount of non-labeled probes clearly disturbed protein-DNA interaction (lanes 3, 4, and lanes 9, 10).

Figure 5 shows the nucleotide sequences of sites of DNA-protein interaction in Probe I and Probe II as determined by DNase-I footprinting. A protected site in Probe I is a 21nts sequence, TTCCTTCTCCCCCCCCCCCCT (from -1,706 to -1,686), that contains a G-string (C or G 12), that is, a $(dG)_{12}$:$(dC)_{12}$ tract, while that in Probe II is a 24nts sequence, TTGGGGAGGGCCCCCCCCCGATCA (from -1,887 to -1,864), also containing a short G-string (C or G 9), that is, a $(dG)_9$:$(dC)_9$ tract. The presence of several other bands with different mobilities on each probe (Fig. 4) suggests the occurrence of multiple DNA-protein interactions in these fragments.

The addition of synthetic poly(dG):poly(dC) blocked complex formation of gastrula nuclear proteins with probes (Fig. 6A, lanes 3, 4 and lanes 9, 10), while plasmid DNA did not compete with the probes for protein binding (Fig. 6A, lanes 5, 6 and lanes 11, 12), indicating the sequence-specific interaction of proteins with G-strings. As shown in Fig. 6B (lanes 3, 4, 7 and 8), the addition of an excess amount of non-labeled Probe I interfered with the interaction of proteins to Probe II, and *vice versa*, suggesting that in spite of difference in the length of G-strings, the natures of DNA-protein interaction at both sites are quite similar.

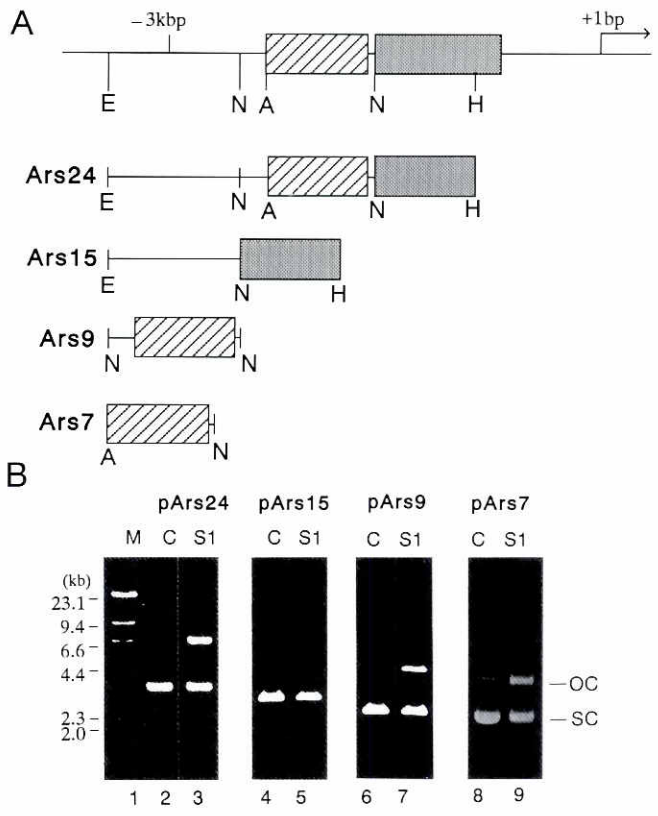


Fig. 2. Structure of the Pyr-Pur region and electrophoretic analysis of S1 nuclease-sensitivity of this region. (A) *Structure of the Pyr-Pur region (uppermost of the panel) and structures of plasmid inserts used in the S1-nuclease assay (Ars24, Ars15, Ars9 and Ars7). Slashed boxes; 622 bp Pyr-Pur region (from -2,078 to -1,457), dotted boxes; AT rich region, A; AvaI, E; EcoRI, H; HindIII, N; NspI (7524)I. An arrow indicates a start site as well as a direction of transcription. Plasmids pArs24* **(lanes 2 and 3)**, *pArs15* **(lanes 4 and 5)**, *pArs9* **(lanes 6 and 7)** *and pArs7* **(lanes 8 and 9)** *were digested with S1 nuclease at pH 5, electrophoresed in 0.7% agarose gels and stained with ethidium bromide. (C) Control without S1 nuclease digestion, S1; plasmids treated with S1 nuclease, M* **(lane 1)**; *lambda DNA digested with HindIII. OC; plasmid in open circular form, SC; plasmid in supercoiled form.*

Discussion

The Pyr-Pur region in the Ars gene of the sea urchin, *Hemicentrotus pulcherrimus*, is featured by the presence of a $(CT)_{11}$:$(GA)_{11}$ tract, $(dC)_{12}$:$(dG)_{12}$ tract and $(dC)_9$:$(dG)_9$ tract. The latter two tracts are known as G-strings and situated at an equal distance from both ends of the $(CT)_{11}$:$(GA)_{11}$ tract. When the plasmids containing the Pyr-Pur region of the Ars gene are negatively supercoiled, the Pyr-Pur region causes a partial strand separation as revealed by the S1-nuclease sensitivity assay. The S1-sensitive sites appear in several clusters. High S1-sensitivity of the 25nts-sequence including 5nts of the 5' half of the $(CT)_{11}$:$(GA)_{11}$

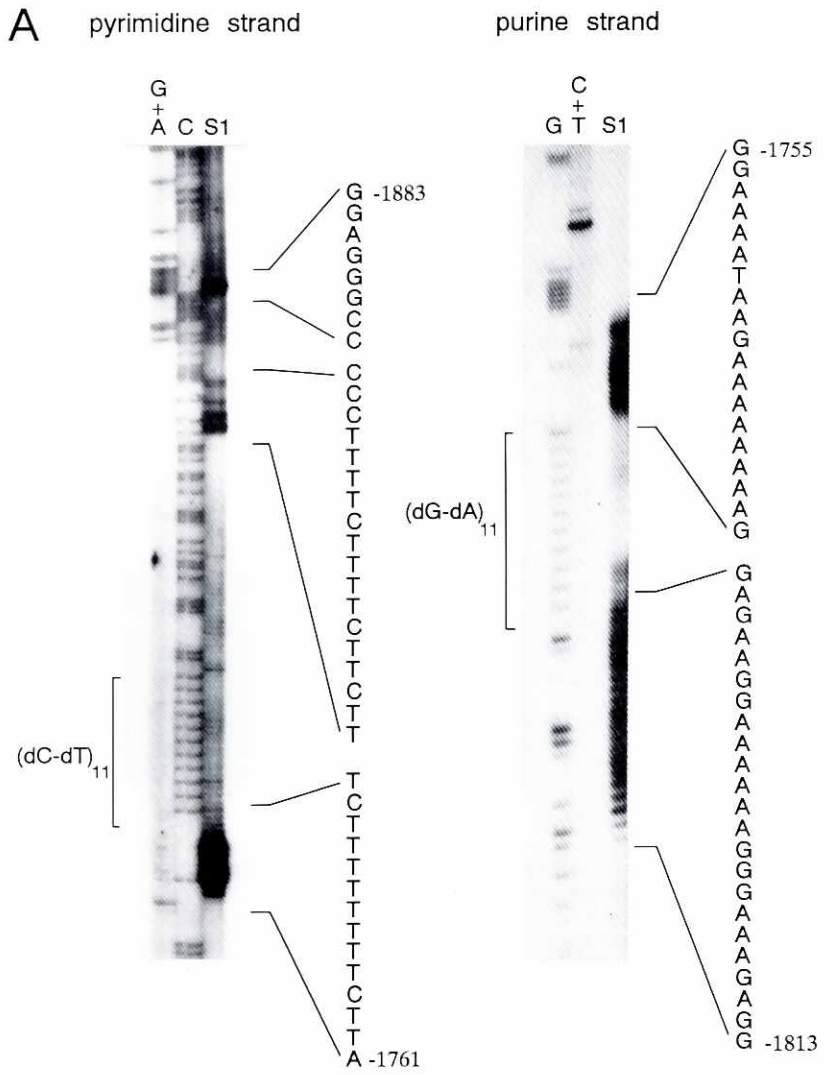
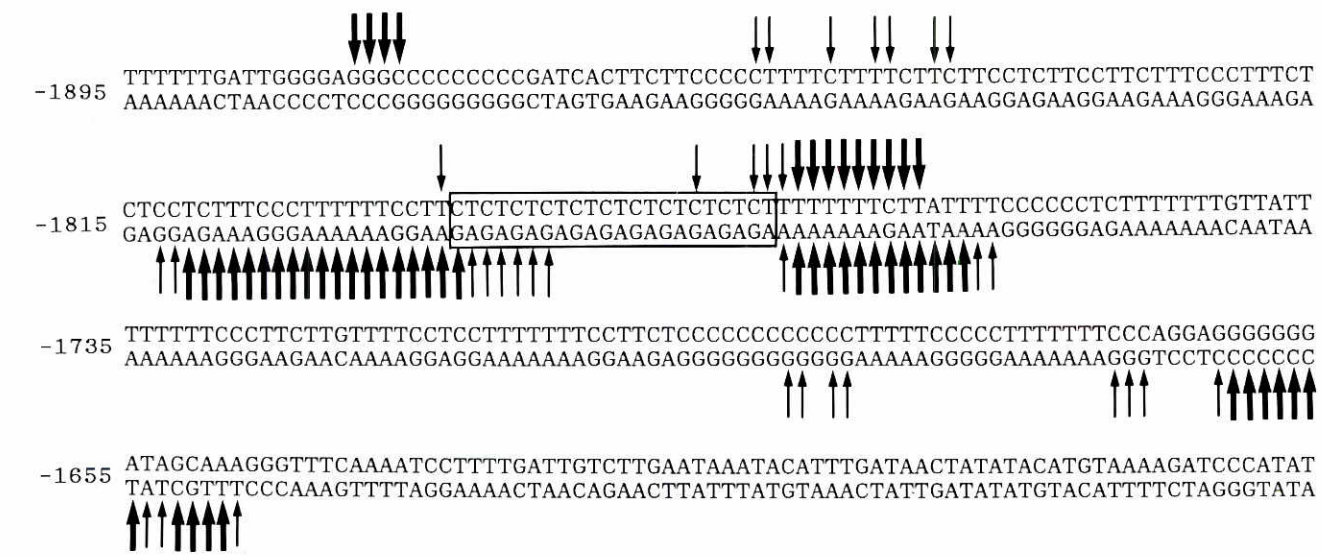


Fig. 3. Determination of S1-nuclease sensitive sites (S1 sites) within Pyr-Pur region and mapping of S1 sites in pyrimidine and purine strands. **(A)** *Supercoiled pArs7 was digested with S1 nuclease and electrophoresed (see Materials and Methods). Lanes G+A, G, C+T and C represent strand sequencings by Maxam and Gilbert reactions and lanes S1 represent S1-nuclease treated Ars7 fragments. S1 sites are shown to the right of each panel.* **(B)** *Schematic presentation of S1 sites. Arrows indicate S1 sites and the arrow sizes reflect the intensities of band staining on the autoradiogram. A* $(CT)_{11}$*:*$(GA)_{11}$ *tract is boxed.*

B



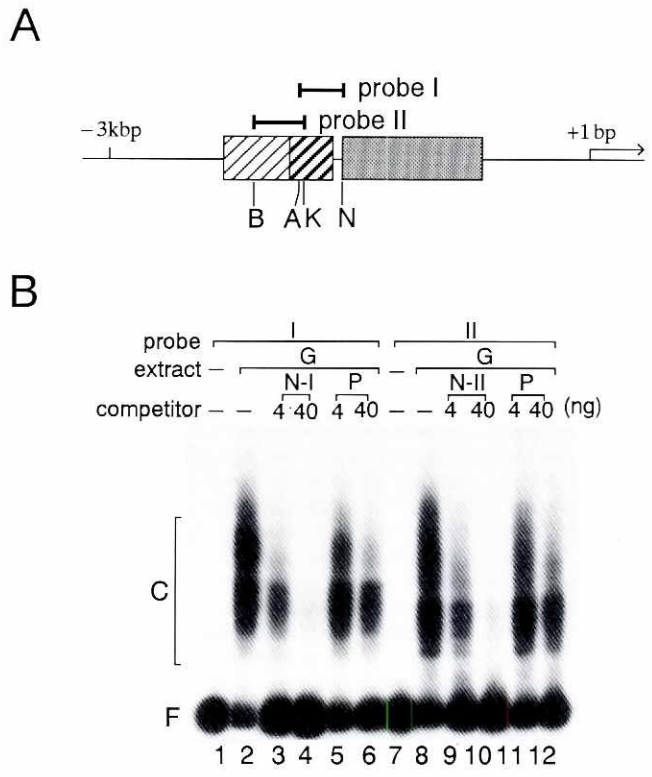


Fig. 4. Mobility shift assay of nuclear proteins interacting with the Pyr-box. (A) *Schematic illustration of the probes used. Probe I is an ApaI-NspI (7524)I 286 bp fragment spanning from -1,880 to -1,595, and probe-II a BspMI-KspI(632)I 274 bp fragment spanning from -2,109 to -1,836. Both probes contain a Pyr-box as illustrated. B, BspM1, Ap; ApaI, K; KspI(632)I, N; NspI(7524)I.* **(B)** *Mobility shift assay of nuclear extracts from gastrula embryos. Probe I* **(lanes 1-6)** *and Probe II* **(lanes 7-12)** *were labeled by fill-in reactions, incubated with gastrula nuclear extracts and electrophoresed. Non-labeled Probe I (N-I) and II (N-II) were used as specific competitors, while the HinfI digest (P) of pUC119 was used as a non-specific competitor. G; gastrula nuclear extract, C; DNA-protein complex, F; free probe.*

tract in the purine strand (Fig. 3B) agrees with a triplex DNA configuration model previously presented by others (Mirkin *et al.*, 1987), assuming that, under the influence of highly negative supercoiling, strand separation occurs at the 5' half of the TTTCCCTTTTTTCCTTCTCTCT in this region, and that the single-stranded pyrimidine strand winds back along a major groove of the double-helical structure of its downstream 3' half forming Hoogsteen base pairings between C and T. This model explains the high S1 sensitivity of the 5' half of the AAAGGGAAAAAAGGAAGAGAGA, a purine counterpart of TTTCCCTTTTTTCCTTCTCTCT. That the Pyr-Pur region loses the S1-nuclease sensitivity when the plasmid is linearized agrees with the Mirkin's model in which a triplex configuration occurred only when the DNA was sufficiently negatively supercoiled (Mirkin *et al.*, 1987).

The presence of several oligo(dA):oligo(dT) tracts in the neighborhood of the $(CT)_{11}$:$(GA)_{11}$ tract also favors the occurrence of an unusual DNA configuration, because DNA is believed to bend at an oligo(dA):oligo(dT) tract (Ohyama and Hashimoto,1989). Recently, it has been reported that polypyrimidine:polypurine sequence in a highly supercoiled plasmid could adopt a triplex DNA configuration even at a neutral pH (Htun and Dahlberg, 1988). It is, therefore, possible that an unusual DNA configuration like a triplex DNA is related to regulation of gene transcription. A recent finding that polypyrimidine:polypurine sequences are mainly localized in the regulatory region as well as in the sites related to recombination (Wells *et al.*, 1988) also supports this hypothesis. Yu and Manley (1986) showed that an S1-sensitive Pyr-Pur sequence present in the adenovirus late promoter has a function to suppress gene transcription. Emerson and Felsenfeld (1984) reported that a G-string in the promoter of the chicken ß-globin gene is related to a conformational change of the promoter that occurs when the gene is actively transcribed.

Also detected in nuclei of the sea urchin embryo were proteins that bind sequence-specifically to G-strings at two distinct sites in the Pyr-box. GC-rich sequences such as G-strings are often detected in promoters of eukaryotic genes and are known to bind multiple positive- or negative-regulator proteins producing the diversity in transcriptional control (Dynan and Tjian, 1983; Briggs *et al.*, 1986; Kageyama and Pastan, 1989). Although the role of G-

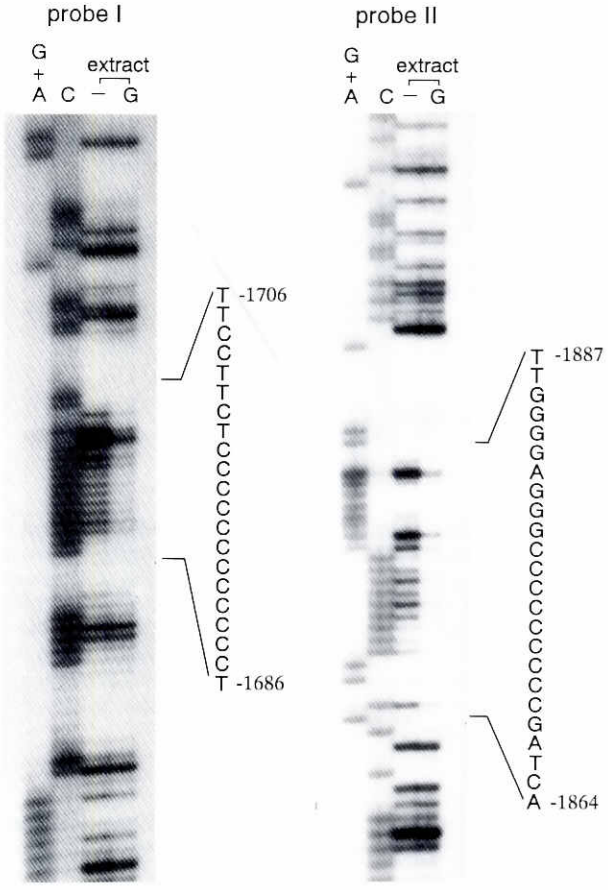


Fig. 5. DNaseI footprinting of the Pyr-box with gastrula nuclear proteins. *Pyrimidine strands of Probe I(I) and Probe II(II) were labeled by fill-in reactions, incubated in the absence(-) or presence(G) of nuclear extract from gastrulae, digested with DNaseI, and electrophoresed (see Materials and Methods). Nucleotide sequences of DNaseI-protected regions are indicated to the right of each panel. G+A and C; Maxam and Gilbert sequencing reactions.*

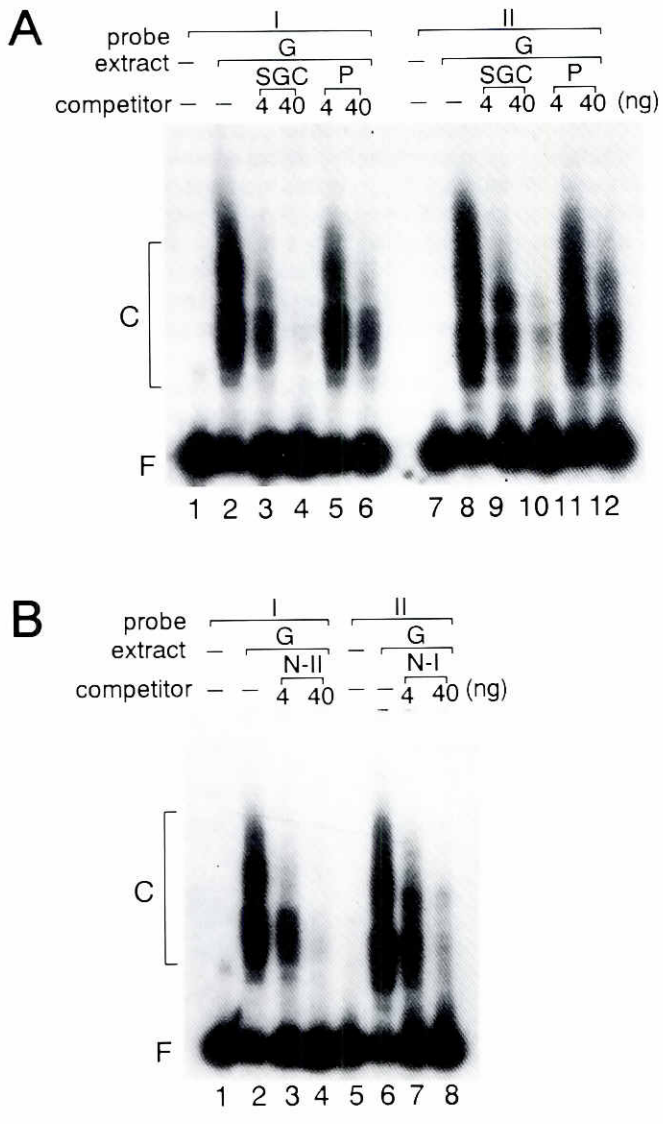


Fig. 6. Mobility shift assay of proteins that specifically bind to G-strings. (A) *Competitive disturbance of protein binding to the probes by synthetic poly(dG)-poly(dC). Probe-I* **(lanes 1-6)** *and Probe-II* **(lanes 7-12)** *were fill-in labeled, and incubated with the gastrula nuclear extract and electrophoresed. SGC; synthetic poly(dG):poly(dC), P; pUC119 HinfI digest(P).* **(B)** *Cross competition between Probe I* **(lanes 1-4)** *and Probe II* **(lanes 5-8)** *for protein binding. Non-labeled Probe I(N-I) and Probe II(N-II) were used as competitor sequences. G; gastrula nuclear extract, C; DNA-protein complex, F; free probe.*

string sequences in regulating the expression of the sea urchin Ars gene is not known, it is of interest to assume that an unusual DNA configuration in the Pyr-box may help binding of regulator proteins to two G-strings that are located at an equal distance (about 75nts) from both ends of the $(CT)_{11}$:$(GA)_{11}$ tract, the site of a possible triplex DNA configuration. Protein binding to two G-strings may in turn stabilize an unusual DNA configuration of the $(CT)_{11}$:$(GA)_{11}$ tract.

Because the distance (about 170 bp) between two G-strings is comparable to that between two nucleosomal units (Felsenfeld, 1992), binding of proteins to G-strings may cause an alteration in a chromatin structure resulting in a change in the level of transcription of the Ars gene. Though our present data on the S1 sites mapping of the Pyr-box that was obtained under an acidic pH agree with a triplex DNA model, experiments under more physiological conditions are definitely needed before reaching any conclusions on DNA configuration taken by the $(CT)_{11}$:$(GA)_{11}$ tract in the Pyr-box and its function in regulating gene transcription during embryogenesis. Further experiments are now in progress in our laboratory.

Materials and Methods

S1 nuclease assay

Four recombinant plasmids, pArs24, pArs15, pArs9 and pArs7, were constructed by inserting various sequences of the Ars 5' flanking region of the sea urchin, *Hemicentrotus pulcherrimus*, into pBluescript (Stratagene). Structures of these plasmids have been schematically depicted in Fig. 2. pArs24 contains all of the *Ars* sequence upstream from the promoter region. pArs15 was constructed by deleting the Pyr-Pur region from the pArs24. pArs9 contains the Pyr-box together with about 170 bp sequence upper from it. pArs7 contains the Pyr-box alone. Four plasmids were treated with S1 nuclease as described by Pulleyblank (Pulleyblank *et al.*, 1985) with slight modifications. Digestion by S1-nuclease was performed for 10 min. at 23°C in 20 μl reaction mixtures containing 50 mM sodium acetate at pH 5, 0.2 mM $ZnCl_2$, 5 μg plasmids and 0.2 unit S1 nuclease, and terminated by addition of 1 mM EDTA. DNA was deproteinized, ethanol-precipitated and electrophoresed on a 0.7% agarose gel.

Fine mapping of S1 nuclease-sensitive sites

Fine mapping of S1 nuclease-sensitive sites in pArs7 was performed according to the method described byNickol and Felsenfeld (1983). To avoid labeling of plasmid sequences, an *Apa*I sequence in the multi-cloning site was deleted from the pArs7 (del-pArs7). For the determination of S1-sensitive sites in the 3' half of the Pyr-Pur box, the del-pArs7 was first digested by either *Apa*I or by *Hind*III. The *Apa*I-digested del-pArs7 was labeled by the 3' end labeling kit (Amersham) and digested with *Hind*III, while the *Hind*III-digested del-pArs7 was filled in with sequenase™ (U.S.B.) in the mixture containing [α^{32} P]dCTP and other dNTPs and digested with *Apa*I. The S1-sensitive sites in the 5' half of the Pyr-Pur box were determined in a similar procedure by using *Apa*I and *Xba*I instead of *Apa*I and *Hind*III. Labeled DNA fragments were precipitated by ethanol and electrophoresed on denaturing 6% polyacrylamide gels.

Preparation of nuclear extracts

Eggs and sperm of the sea urchin, *Hemicentrotus pulcherrimus*, were collected by artificial spawning by KCl. Inseminated eggs were grown in the filtered sea water at 20°C with constant stirring, and the embryos or larvae were harvested by centrifugation (Sasaki *et al.*, 1988). Nuclear extracts were prepared as described by Calzone (Calzone *et al.*, 1988), and extracts were loaded onto a DEAE cellulose column equilibrated with buffer C (20 mM Hepes at pH 7.9, 40 mM KCl, 0.1 mM EDTA, 10 mM DTT, 20% glycerol). Proteins were eluted from the column with buffer C containing 0.4 M KCl, and their binding to the Pyr-Pur fragment was monitored by mobility shift assay. The active fraction was stored at -80°C until use.

Mobility shift assay

Mobility shift assay was performed as described by Calzone (Calzone *et al.*, 1988). The probe fragments (Fig. 4) were subcloned by inserting them into the *Eco*RIsites of pBluescript and excised from the plasmids by *Bam*HI and *Pst*I, and their 5' overhangs were filled in by use of [^{32}P]dCTP, other dNTPs and sequenase™ (U.S.B). Labeled probes were mixed with gastrula nuclear extracts in 10 μl of the binding buffer (20 mM Hepes at pH 7.9, 120 mM KCl, 5 mM $MgCl_2$ and 10 mM DTT) containing poly(dI-dC). After incubating the mixture for 15 min at 13°C, 1 μl of the sample buffer consisting of 15% Ficoll-400, 0.25% bromophenol blue and 0.25% xylene

cyanol was added. Electrophoresis was performed on a 4% acrylamide gel at 4°C, and the gel was dried to visualize bands by autoradiography. For competition experiments, 4-40 ng of a specific competitor [non-labeled probe or poly(dG)-poly(dC) (Pharmacia)] or a non-specific competitor [*Hinf*I-digests of pUC119] was added to the reaction mixture prior to addition of the nuclear extract.

DNase-I footprinting

DNase-I footprinting was performed as described by Barberis (Barberis *et al.*, 1987). Pyrimidine strands of Probe I and II (Fig. 4) were labeled as described in mobility shift assay. Labeled DNA fragments were incubated with gastrula nuclear extracts under a condition described for the mobility shift assay. DNase-I treatment was carried out in the presence of 1.5 mM $MgCl_2$ and 1-5 units of DNase-I, and terminated by addition of 0.6 M NaCl solution containing 0.2% sodium dodecylsulfate and 10 mM EDTA. DNA was deproteinized and ethanol-precipitated, suspended in the denaturing buffer consisting of 80% formamide, 10 mM NaOH, 1 mM EDTA, 0.1% bromophenol blue and 0.1% xylene cyanol, and electrophoresed on denaturing 6% acrylamide gels.

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References

AKASAKA, K. and TERAYAMA, H. (1983). Sulfated glycan present in the EDTA extract of *Hemicentrotus* embryos (midgastrula). *Exp. Cell Res. 146:* 177-185.

AKASAKA, K., AMEMIYA, S. and TERAYAMA, H. (1990a). Scanning electron microscopical study of the inside of sea urchin embryos *(Pseudocentrotus depressus). Exp. Cell Res. 129:* 1-13.

AKASAKA, K., UEDA, T., HIGASHINAKAGAWA, T., YAMADA, K. and SHIMADA, H. (1990b). Spatial patterns of arylsulfatase mRNA expression in sea urchin embryo. *Dev. Growth Differ. 32:* 9-13.

AKASAKA, K., YAMAMOTO, T., SAKAMOTO, N., MOROKUMA, J., TAKATA, K., EGUCHI, S. and SHIMADA, H. (1994). Corrected nucleotide sequence of 5'flanking region of Arylsulfatase gene of the sea urchin, *Hemicentrotus pulcherrimus,* and expression of Ars-luciferase fusion constructs transferred into sea urchin embryos. *Dev. Growth Differ. 36(4).* (In press).

BARBERIS, A., SUPERTI-FURGA, G. and BUSSLINGER, M. (1987). Mutually exclusive interaction of the CCAAT-binding factor and of displacement protein with overlapping sequences of a histone gene promoter. *Cell 50:* 347-359

BRIGGS, M.R., KADONAGA, J.T., BELL, S.T. and TJIAN, R. (1986). Purification and biological characterization of the promoter-specific transcription factor, Sp1. *Science 234:* 47-52.

CALZONE, F.J., THEZE, N., THIEBAUD, P., HILL, R.L., BRITTEN, R.J. and DAVIDSON, E.H. (1988). Development appearance of factors that bind specifically to *cis*-regulatory sequences of a gene expressed in the sea urchin embryo. *Genes Dev. 2:* 1074-1088.

CHRISTOPHE, D., CABRER, B., BACOLLA, A., TARGOVNIK, H., POHL, V. and VASSART, G. (1985). An unusually long poly(purine)-poly(pyrimidine) sequence is located upstream from the human thyroglobulin gene. *Nucleic Acids Res. 13:* 5127-5144.

DYNAN, W.S. and TJIAN, R. (1983). The promoter-specific transcription factor Sp1 binds to upstream sequences in the SV40 early promoter. *Cell 35:* 79-87.

EMERSON, B.M. and FELSENFELD, G. (1984). Specific factor conferring nuclease hypersensitivity at the 5' end of the chicken adult ß-globin. *Proc. Natl. Acad. Sci. USA 81:* 95-99.

EVANS, T. and EFSTRATIADIS, A. (1986). Sequence-dependent S1 nuclease hypersensitivity of a heteronomous DNA duplex. *J. Biol. Chem. 261:* 14771-14780.

FELSENFELD, G. (1992). Chromatin as an essential part of the transcriptional mechanism. *Nature 355:* 219-224.

FINER, M.H., AHO, S., GERSTENFELD, L.C., BOEDTKER, H. and DOTY, P. (1987). Unusual DNA sequences located within the promoter region and the first intron of the chicken pro-α1(I) collagen gene. *J. Biol. Chem. 262:* 13323-13332.

FOWLER, R.F. and SKINNER, D.M. (1986). Eukaryotic DNA diverges at a long and complex pyrimidine-purine tract that can adopt altered conformations. *J. Biol. Chem. 261:* 8994-9001.

GILMOUR, D.S., THOMAS, G.H. and ELGIN, S.C.R. (1989). *Drosophila* nuclear proteins bind to regions of alternating C and T residues in gene promoters. *Science 245:* 1487-1490.

HENTSCHEL, C.C. (1982). Homocopolymer sequences in the spacer of a sea urchin histone gene repeat are sensitive to S1 nuclease. *Nature 295:* 714-716.

HTUN, H. and DAHLBERG, J.E. (1988). Single strands, triple strands, and kinks in H-DNA. *Science 241:* 1791-1796.

HTUN, H., LUND, E. and DAHLBERG, J.E. (1984). Human U1 RNA genes contain an unusually sensitive nuclease S1 cleavage site within the conserved 3' flanking region. *Proc. Natl. Acad. Sci. USA 81:* 7288-7292.

KAGEYAMA, R. and PASTAN, I. (1989). Molecular cloning and characterization of a human DNA binding factor that represses transcription. *Cell 59:* 815-825.

KOHWI-SHIGEMATSU, T., GELINAS, R. and WEINTRAUB, H. (1983). Detection of an altered DNA conformation at specific sites in chromatin and supercoiled DNA. *Proc. Natl. Acad. Sci. USA 80:* 4389-4393.

LEWIS, C.D., CLARK, S.P., FELSENFELD, G. and GOULD, H. (1988). An erythrocytes-specific protein that binds to the poly(dG) region of the chicken ß-globin gene promoter. *Genes Dev. 2:* 863-873.

MACE, H.A.F., PELHAM, H.R.B. and TRAVERS, A.A. (1983). Association of an S1 nuclease-sensitive structure with short direct repeats 5' of *Drosophila* heat shock genes. *Nature 304:* 555-557.

McKEON, C., SCHMIDT, A. and CROMBRUGGE, B. (1984). A sequence conserved in both promoter region and the first intron of the chicken pro-α2(I) collagen gene. *J. Biol. Chem. 259:* 6636-6640.

MIRKIN, S.M., LYAMICHEV, V.I., BELOTSERKOVSKII, B.P. and FRANK-KAMENETSKII, M.D. (1987). DNA H form requires a homopurine-homopyrimidine mirror repeat. *Nature 330:* 495-497.

NICKOL, J.M. and FELSENFELD, G. (1983). DNA conformation at the 5' end of the chicken ß-globin gene. *Cell 35:* 467-477.

OHYAMA, T. and HASHIMOTO, S. (1989). Upstream half of adenovirus type 2 enhancer adopts a curved DNA conformation. *Nucleic Acids Res. 17:* 3845-3853.

PANAYOTATOS, N. and WELLS, R.D. (1981). Cruciform structures in supercoiled DNA. *Nature 289:* 466-470.

PULLEYBLANK, D.E., HANIFORD, D.B. and MORGAN, A.R. (1985). A structural basis for S1 nuclease sensitivity of double stranded DNA. *Cell 42:* 271-280.

RAPRAEGER, A.C. and EPEL, D. (1981). The appearance of an extracellular arylsulfatase during morphogenesis of the sea urchin, *Strongylocentrotus prupuratus. Dev. Biol. 88:* 269-279.

SASAKI, H., AKASAKA, K., SHIMADA, H. and SHIROYA, T. (1987a). Purification and characterization of arylsulfatase from sea urchin *(Hemicentrotus pulcherrimus). Comp. Biochem. Physiol. 88B:* 147-152.

SASAKI, H., AKASAKA, K., SHIROYA, T. and SHIMADA, H. (1987b). Developmental timing of synthesis and translation of arylsulfatase mRNA in sea urchin embryo. *Dev. Growth Differ. 29:* 317-322.

SASAKI, H., YAMADA, K., AKASAKA, K., KAWASAKI, H., SUZUKI, K., SAITO, A., SATO, M. and SHIMADA, H. (1988). cDNA cloning, nucleotide sequence and expression of the gene for arylsulfatase in the sea urchin *(Hemicentrotus pulcherrimus)* embryo. *Eur. J. Biochem. 177:* 9-13.

SINGLETON, C.K., KLYSIK, J., STIRDIVANT, S.M. and WELLS, R.D. (1982). Left-handed Z-DNA is induced by supercoiling in physiological ionic conditions. *Nature 299:* 312-316.

WELLS, R.D. (1988). Unusual DNA structures. *J. Biol. Chem. 263:* 1095-1098.

WELLS, R.D., COLLIER, D.A., HANVEY, J.C., SHIMIZU, M. and WOHLRAB, F. (1988). The chemistry and biology of unusual DNA structures adopted by oligopurine-oligopyrimidine sequences. *FASEB J. 2:* 2923-2949.

WINTER, E. and VARSHAVSKY, A. (1989). A DNA binding protein that recognizes oligo(dA)-oligo(dT) tracts. *EMBO J. 8:* 1867-1877.

YANG, Q., ANGERER, L.M. and ANGERER, R.C. (1989). Structure and tissue-specific developmental expression of a sea urchin arylsulfatase gene. *Dev. Biol. 135:* 53-67.

YU, Y.T. and MANLEY, J.L. (1986). Structure and function of the S1 nuclease-sensitive sites in the adenovirus late promoter. *Cell 45:* 743-751.