Volume 17 Number 15 1989 Nucleic Acids Research

The 5S rRNA-histone repeat in the crustacean *Artemia:* structure, polymorphism and variation of the 5S rRNA segment in different populations

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Received May 2, 1989; Revised and Accepted July 14, 1989 EMBL accession nos X14815–X14817 (incl.)

ABSTRACT

5S rRNA genes are linked to the histone genes in the 13 populations of the crustacean *Artemia* that we have studied. In all cases, two types of repeat units are found. Southern blot analysis of all populations shows that they can be grouped into three classes: a) American bisexuals; b) Eurasian bisexuals, and c) parthenogenetic organisms (all from Eurasia). Restriction analysis of a bisexual population from San Francisco Bay shows that the two repeat units are of 9.0 and 8.5 kb (with minor heterogeneities of restriction sites). In parthenogenetic organisms, the two repeat units are of approximately 12 kb. Sequencing data from the region of the 5S rRNA from the San Francisco Bay population, shows that in both types of units, the single 5S rRNA gene (315 bp in length), is located 430 bp downstream the 3' regulatory sequences of the H2A gene, the last gene in the histone cluster. We have isolated three clones that contain 5S rRNA sequences. Two of them (one from an American bisexual and the other from a parthenogenetic population) contain histone and 5S rRNA genes, both with the same transcriptional polarity. The third clone, lacking histone genes, is likely to be an orphon derived from the parthenogenetic population.

INTRODUCTION

The structure of the 5S rRNA genes has been studied in a wide variety of eukaryotic organisms. 5S rRNA genes are, in general, organized in tandem arrays constituting a repeated gene family. The intergenic regions range from 2 kb in the Syrian hamster (1) to 375 bp in *Drosophila melanogaster* (2). The exceptions to this rule are lower eukaryotes, like yeasts and the slime mold *Dictyostelium discoideum* (3) and the copepod *Calanus* (4), where the 5S rRNA genes are linked to the large rRNA genes, or scattered throughout the genome, as in *Neurospora crassa* (5).

Our first characterization of a 5S rRNA gene in the crustacean *Artemia* showed that a single copy of this sequence was found in the middle of a 5.5 kb-long region (6). Subsequent studies by Bagshaw and coworkers and our group (7-10) have demonstrated that 5S rRNA genes and histone genes are linked, although no conclusions were obtained on whether this linkage was a peculiarity of the San Francisco Bay population and not present in other *Artemia* strains, or if it was a particular case for *Artemia* and not found in other systems.

In this paper, we demonstrate the linkage of 5S rRNA and histone genes by

sequencing the 5S rRNA gene region of two clones from different populations. We also demonstrate that these genes are formed in two different organizations, and that both characteristics are common to all the populations of *Artemia* we have studied. Crustaceans is a group of organisms that are very poorly studied at the genomic level. Our results could help to fill this gap in a gene family very well suited for evolutionary studies.

MATERIALS AND METHODS

Organisms and DNA.

Artemia cysts were purchased from San Francisco Bay Brand, Inc. Batch 1808 was a population from San Francisco Bay; other batch without number was purchased in 1982 to the same supplier and contained a parthenogenetic population of Chinese origin. Cysts from the other different populations were a kind gift of Dr. F. Amat (Instituto de Acuicultura del CSIC, Castellón, Spain). High molecular weight DNA was purified as described by Cruces *et al.* (11), with minor modifications.

Plasmids and probes

cDm500 (12) contains a whole histone repeat from *D. melanogaster* and was provided by Dr. D. Hogness, from Stanford University. pArt5H-a (referred to as pMD59 by Díaz-Guerra *et al.* (6)) was obtained by cloning *Pst*I-digested *Artemia* DNA into the corresponding site of pBR322. pArt5H-b and pArt5-b were isolated from the genotheque described by Gallego *et al.* (13). Recombinant phages were selected by hybridization with the *Rsa*I fragment (positions 25 to 75) of the 5S rRNA (obtained from pArt5H-a). The inserts of the positive phages were subcloned into the *Sal*I site of pUC9.

DNA sequencing

To sequence pArt5H-a, the plasmid was digested with *Hin*dIII to obtain the 3' end of the histone cluster; the 362 bp *Hin*dIII fragment (positions 1 to 362 in Fig. 5) was subcloned in pUC18 and sequenced as described by Chen and Seeburg (14), obtaining the whole sequence of the fragment from both universal primers. To sequence the 5S rRNA gene and surrounding regions, pArt5H-a was digested with *Hin*dIII plus *Hin*cII (positions 362 to 1066 in Fig. 5, respectively). This fragment was subcloned in pUC9 and sequenced by the chemical method (15) from both ends and from the *Bst*EII site (position 851 in Fig. 5) in both directions. We have not an overlapping fragment between the *Hin*dIII and the *Hin*dIII/*Hin*cII fragments in pArt5H-a. We consider, however, that the similarity of this region with that of pArt5H-b makes very unlikely the presence of a small *Hin*dIII fragment between them.

To sequence pArt5H-b, the plasmid was digested with *Bst*EII and *Hpa*I (see Fig. 4). The *Bst*EII fragment (positions 1 to 945 in Fig. 5) was sequenced by the dideoxy

Table 1
Restriction fragments hybridizing to histone or 5S rRNA probes in genomic *Artemia* DNA

	Hybridizing fragments	
Enzyme(s)	Histones	5S RNA
*Xho*I	>25; 8.5	>25; 8,5.
*Pst*I	8.5; 5.5	8.5; 5.5
*Xho*I+*Pst*I	5.5; 4.5; 4.0	5.5; 4.5
*Bgl*I	6.0; 5.1; 3.4; 3.0	5.1; 3.0
*Xba*I	9.0; 4.9; 3.6	9.0; 4.9
*Bgl*I+*Xba*I	5.1; 4.1; 3.0; 1.1; 0.9	4.1; 3.0
*Bam*HI	9.0	9.0
*Sal*I	>25; 9.0	>25; 9.0
*Bam*HI+*Sal*I	8.5; 6.5; 2.5	8.5; 2.5
*Pvu*II	>25; 4.8; 4.2	>25; 4.2
*Bst*EII	6.6; 5.9; 2.4; 1.5; 0.9	2.4; 0.9
*Pvu*II+*Bst*EII	5.9; 4.8; 2.4; 1.5; 0.9; 0.75	2.4; 0.9
*Hin*dIII	7.9; 6.5; (5.9); (2.7); 2.1; 0.45	7.9; (2.7); 2.1
*Hin*dIII+*Xho*I	6.5; (5.9); 4.5; 3.4; (2.7); 2.1; 0.45	4.5; (2.7); 2.1
*Eco*RI	>25; (9.0); 7.8	>25; (9.0); 7.8
*Bgl*II	>25; 9.0	>25; 9.0
*Eco*RI+*Bam*HI	9.0; (5.5); (3.5)	9.0; (3.5)
*Bgl*II+*Xba*I	9.0; (7.9); 3.6; 2.5; 1.2	9.0; (7.9); 2.5

The size of the fragments is expressed in kb.

technique (14). The *Bst*EII-*Hpa*I fragment was sequenced from the BstEII site by the dideoxy technique, and also from the EcoRI sites (positions 1074 and 1109 in Fig. 5) in both directions by the chemical method. pArt5-b was sequenced by the chemical method from the BstEII site (position 308 in Fig. 5). In every case, more than one gel was read from each region.

Alignment of the sequences was done using the Nucaln program of Lipman and Wilbur (16).

Other methods

All conventional methods of DNA manipulation were done according to the original protocols or as described in the Manual of Maniatis *et al.* (17).

RESULTS

Organization of 5S rRNA-histone genes

Histone and 5S rRNA genes in *Artemia* are repeated families, with 100 copies per haploid genome (8). Restriction analysis indicates that these genes are arranged in tandem, as happens in other eukaryotic organisms. We have carried out this type of analysis with Southern blots by using ten restriction endonucleases and mixtures of them, and hybridizing with histone or 5S rRNA probes (Table 1). Representative digests are shown in Fig. 1. The results have two common characteristics: 1) all of the fragments detected by the 5S rRNA probe are also detected by the histone probe. 2) In some digestions, besides discrete fragments, there are high molecular weight fragments (larger than the 23 kb marker of the l DNA digested with *Hin*dIII).

The 5S rRNA probe does not contain any sites for the restriction endonucleases used (shown in Table 1); it must, therefore, detect single fragments. The fact that in every digestion tested there are two fragments is indicative of two types of organization for the 5S rRNA genes. As mentioned before, all 5S rRNA-positive fragments are also detected by the histone probe, although this probe detects fragments not shown with the 5S rRNA probe.

These data, together with the restriction analysis of the plasmid pArt5H-a (the 5.5 kb *Pst*I fragment of one American bisexual repeat, see below), and several more different double digestions not shown (using as probes fragments derived from pArt5H-a), have allowed us to construct the physical maps shown in Fig. 2. These maps show that histone and 5S rRNA genes are linked and that there are two types of repeat units, of 9.0 and 8.5 kb, in the population of San Francisco Bay. The maps also explain the presence of non-defined high molecular weight fragments that disappear in double digestions. In the example of Fig. 1, the *Xho*I digest shows a fragment of 8.5 kb and high molecular weight fragments. In the *Pst*I-*Xho*I double digest both type of fragments are cut, giving rise to two (with the 5S rRNA probe) or three (with the histone probe) fragments. As shown in Fig. 2, type I repeats have no *Xho*I sites, whereas type II repeats have one site, giving a unit length fragment of 8.5-kb.

The maps shown in Fig. 2 are for the most abundant repeats. We have observed restriction site polymorphisms, mainly in type I repeats, as those depicted for *Bgl*II, *Hin*dIII and *Eco*RI (these polymorphic fragments are shown between brackets in Table 1). The relative intensities of the two bands of the *Pst*I digest shown in Fig. 1 indicate that type I is the most abundant, about 60% of the total.

Heterogeneity of 5S rRNA-histone genes in different *Artemia* populations

We have already shown that different *Artemia* populations have differences in the organization of some genes. Satellite I is present only in American populations

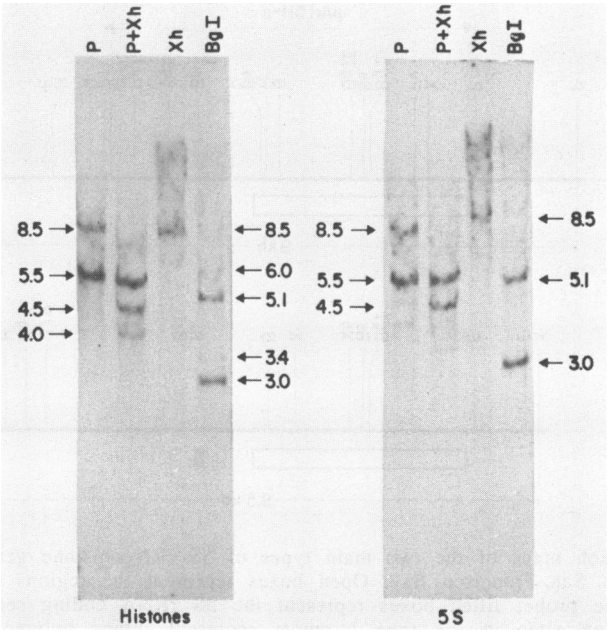


Fig. 1. Southern analysis of 5S rRNA and histone genes from *Artemia* from San Francisco Bay. DNA (five mg) was digested with the different restriction endonucleases and hybridized with histones or 5S rRNA probes as indicated. Numbers refer to size of the detected fragments in kb. P, *Pst*I; Xh, *Xho*I; BgI, *Bgl*I.

(18, 19). rRNA genes, also, can be found in two types of repeats, one found in American populations and the other found in parthenogenetic animals from Eurasia (13, 20).

To examine whether this kind of heterogeneity is also observed in the 5S rRNA-histone genes, we did Southern blot analysis of 13 different populations of *Artemia*, from different origins and with different types of reproduction. Fig. 3 shows examples of these experiments. Parthenogenetic populations (first three lanes) apparently give only one *Bgl*I fragment of approximately 12 kb when hybridized with the 5S rRNA probe. The same results have been obtained with the other parthenogenetic populations used: Delta del Ebro, Calpe and Ayamonte (all from Spain), and Alcochete (Portugal). Although this result could be interpreted as being only one type of repeat in parthenogenetic *Artemia*, the digests shown in part B of the Figure (Delta del Ebro) indicate that the 5S rRNA probe detects two types of repeat units in these organisms (as there is no target for *Eco*RI, *Xba*I and *Pvu*II in the probe). Moreover, the similar intensity of the fragments rules out the possibility of one being a minor polymorphism.

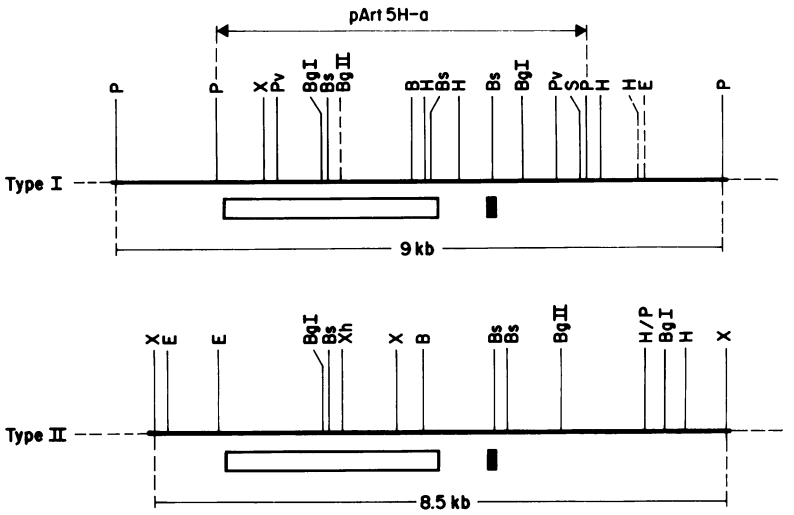


Fig. 2. Restriction maps of the two main types of 5S rRNA-histone genes present in *Artemia* from San Francisco Bay. Open boxes represent the regions that hybridize with the histone probe; filled boxes represent the 5S rRNA coding sequence. The dashed restriction sites in the type I repeat represent minor polymorphisms. Restriction endonucleases are: B, *Bam*HI; BgII, *Bgl*II; Bs, *Bst*EII; E, *Eco*RI; H, *Hin*dIII; Pv, *Pvu*II; S, *Sal*I; X, *Xba*I. Other symbols are as in Fig. 1.

Lanes four and five correspond to bisexual populations from Spain. In this case, the 5S rRNA probe again detects two *Bgl*I fragments, of 4.4 and 4.0 kb (besides a partially digested one of approximately 6.7 kb). We also tested American bisexual populations, different from the one from San Francisco Bay used in the experiment shown in Table 1 and Fig. 1. Besides the two shown in Fig. 3, *Artemia* from Yucatán (México) gave also the same results, that is two fragments are of 5.1 and 3.0 kb, in agreement with the maps shown in Fig. 2.

Although not applicable to the parthenogenetic populations, we have ruled out that the presence of two types of repeat units could be due to sexual dimorphism; experiments done with male and female individuals gave the same results (data not shown).

As our main interest was to demonstrate that 5S rRNA and histone genes were linked, and also due to the complexity of the work involved, we have not tried to construct a physical map for the parthenogenetic or bisexual populations from Eurasia (although pArt5H-b contains a 9.0 kb *Sal*I fragment from a parthenogenetic population, see below). We have not found restriction endonucleases that cut only once in the Euroasiatic bisexual organisms, so we cannot establish their repeat unit length. We have these data, however, for the other two types of populations:

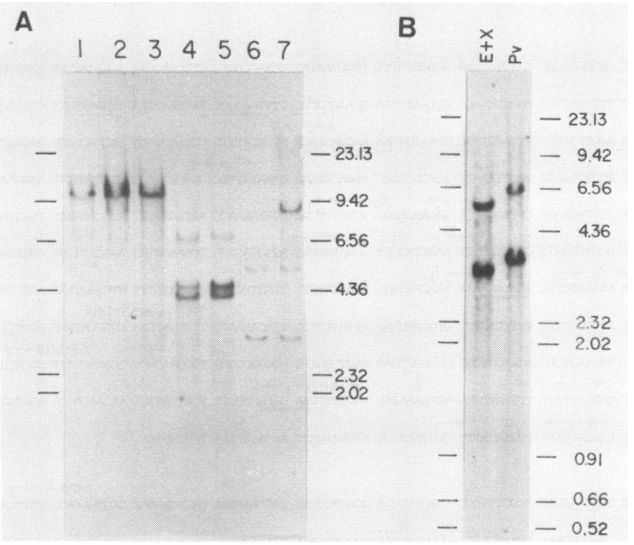


Fig. 3. Southern analysis of 5S rRNA-histone genes from different *Artemia* populations. For each digest, five mg of DNA were used. Blots were hybridized in all cases with the 5S rRNA probe. In part A, DNAs were digested with *BglI* and in part B, with *Eco*RI plus *Xba*I (E+X) or *Pvu*II (Pv). Numbers refer to marker DNAs in kb. A, DNAs from: lane 1, Sanlúcar de Barrameda (Spain, parthenogenetic, diploid); lane 2, Tianjin (China, parthenogenetic, tetraploid); lane 3, Saelices (Spain, parthenogenetic, tetraploid); lane 4, San Fernando (Spain, bisexual); lane 5, Bonmatí (Spain, bisexual); lane 6, Great Salt Lake Utah (USA, bisexual); lane 7, Boca Chica (Venezuela, bisexual). In part B, Delta del Ebro (Spain, parthenogenetic, tetraploid).

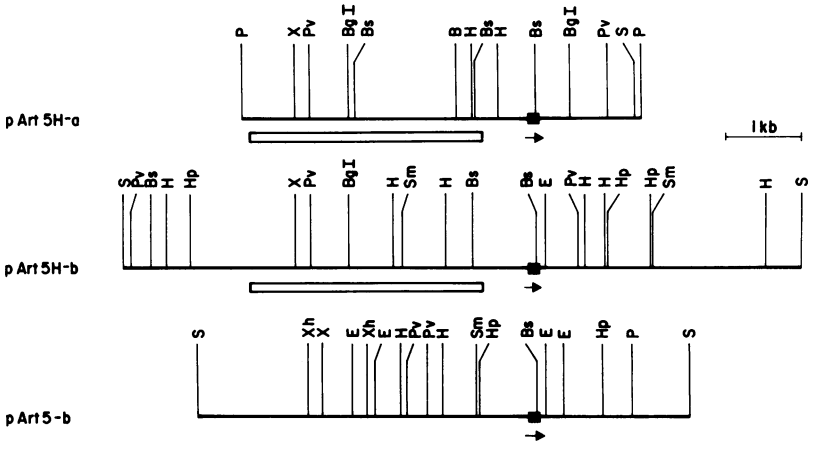
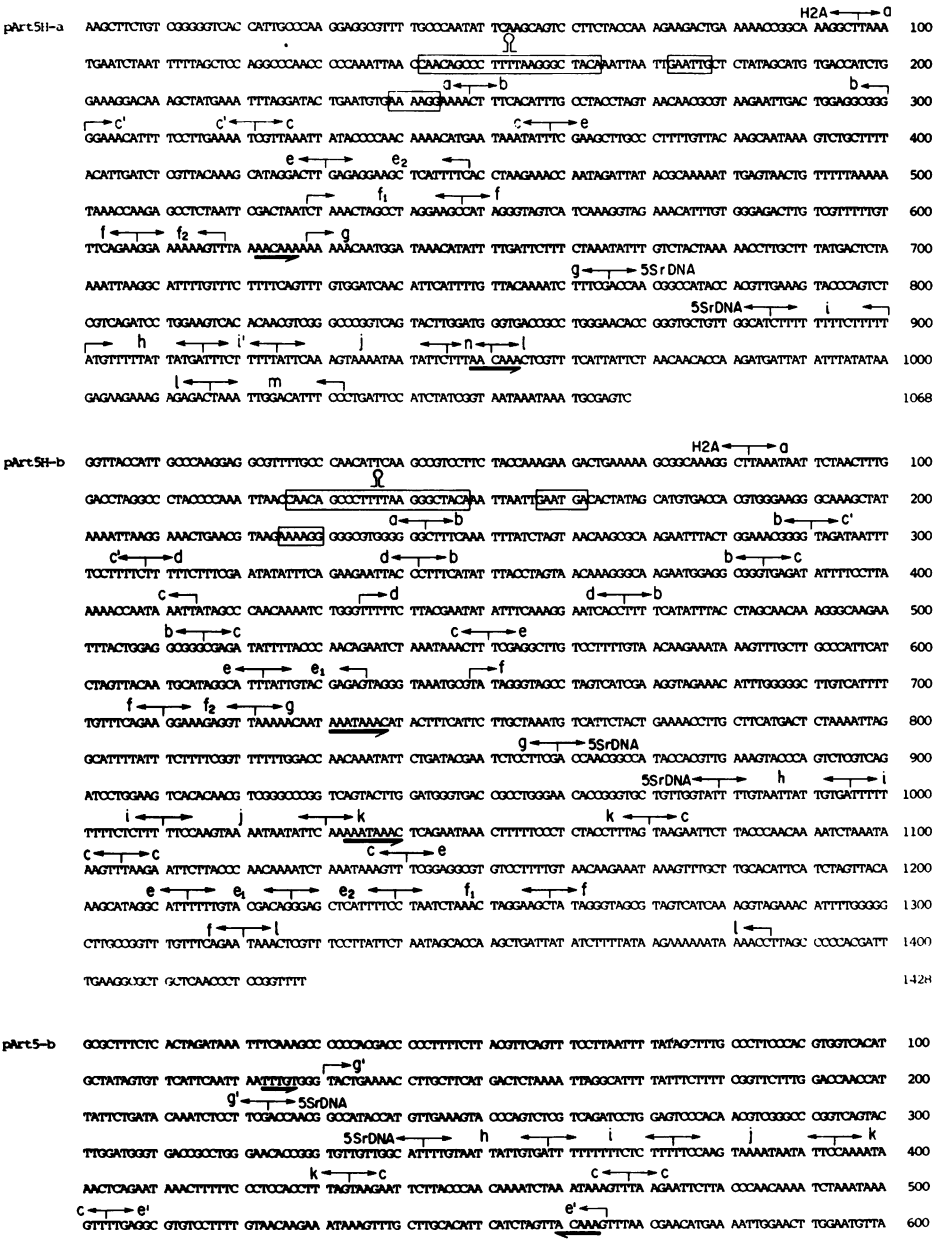


Fig. 4. Restriction map of plasmids pArt5H-a, pArt5H-b and pArt5-b. Boxes are the same as in Fig. 2. Arrow indicates the direction of transcription of the 5S rRNA gene. Symbols are the same as in Fig. 2 and 3, plus Hp, *Hpa*I; Sm, *Sma*I.


pArt5H-a
pArt5H-b
pArt5-b
H2A
5SrDNA

American bisexuals have two repeat units, of 9.0 and 8.5 kb, whereas parthenogenetic populations have two different repeat units (independently of ploidy), but both with a similar length of approximately 12 kb.

Structure of 5S rRNA-histone genes

We have isolated three clones that hybridize with 5S rRNA. One of them, pArt5H-a, was obtained from an American bisexual, as mentioned before. The *Artemia* DNA used to generate the other two clones, pArt5H-b and pArt5-b was isolated from a commercial batch with a parthenogenetic population, most probably from China (13). The restriction maps of the three plasmids are shown in Fig. 4.

The maps shown in Figures 2 and 4 outline the 5S rRNA-histone repeat unit: there is only one 5S rRNA gene, of approximately 315 bp (see below) per repeat. The histone gene cluster is of 3.2 kb and is located 430 bp 5' of the 5S rRNA gene.

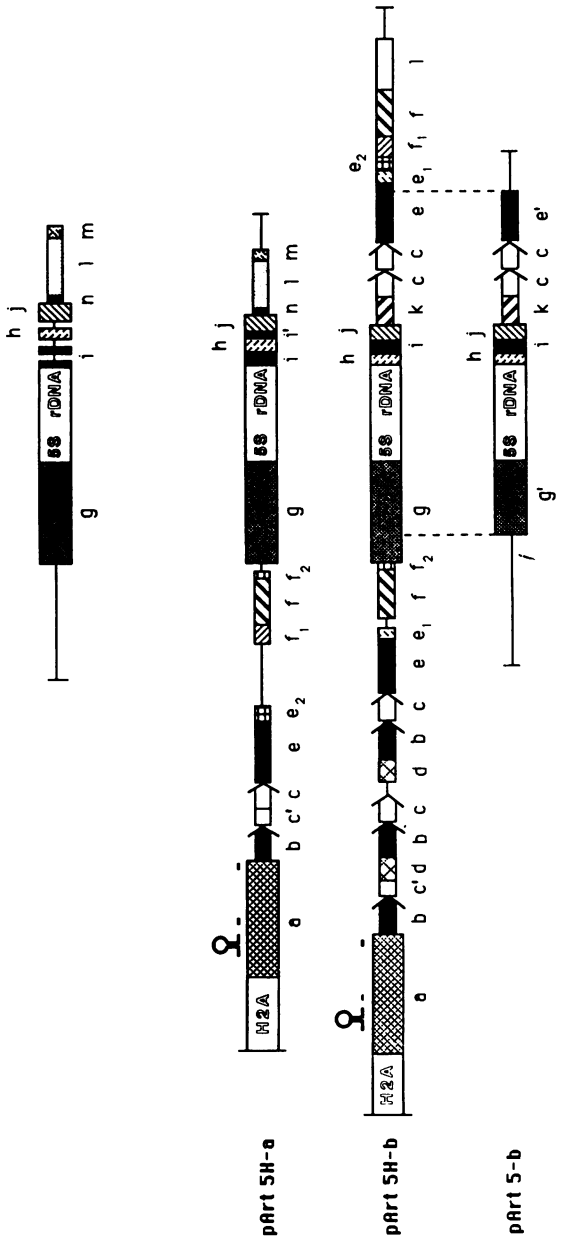
The restriction map of pArt5H-a corresponds to that of the 5.5 kb *Pst*I fragment of the type I repeat shown in Fig. 2. The map obtained for pArt5H-b does not coincide with those of Fig. 2 because it is of parthenogenetic origin. This is further confirmed by the fact that its map agrees with the digestions shown in Fig. 3B: the 3.8 kb fragment of *Pvu*II and the 3.5 kb fragment of the *Eco*RI plus *Xba*I double digest are present in pArt5H-b. Therefore, pArt5H-a is the 5.5 kb *Pst*I fragment of the type I repeat from American bisexuals; pArt5H-b is a 9.0 kb *Sal*I fragment of one of the repeat units from parthenogenetic populations.

Analysis of the sequences surrounding the 5S rRNA genes

We have sequenced 1068 bp in pArt5H-a and 1428 bp in pArt5H-b. In both cases, the sequence goes from the 3' end of the histone cluster to beyond the 5S rRNA gene. The 653 bp sequenced from pArt5-b include only the 5S rRNA gene (Fig. 5).

The sequences in Fig. 5 show that these intergenic spacers are made of small, very related, regions, with a similarity between them from 79 to 95%. These regions are shown schematically in Fig. 6. The organization of the sequence is similar in pArt5H-a and pArt5H-b. We think that pArt5-b can be classified as an orphon derived from a cluster like that represented by pArt5H-b, as defined by Childs *et al* (21): there is no relationship of the sequences surrounding the 5S rRNA gene, and even more, regions "g" and "e" are truncated. In the boundaries of the orphon there is an

Fig. 5. Sequence of the region that contains the 5S rRNA gene in plasmids pArt5H-a, pArt5H-b and pArt5-b. Letters above the sequences indicate regions of similarity between different plasmids. Superscripts denote that the regions are truncated. Subscripts denote related parts of a given region. The 3' regulatory sequences for H2A genes are boxed. 'Ω' represent the hairpin structure located in the first regulatory sequence. Arrows below the sequence indicate the different direct or inverted repeats discussed in the text.


h j
i n l m
5S rDNA
g
h j
i i' n l m
5S rDNA
g
f1 f f2
a b c' c e e2
M2A
pArt 5H-a
h j
i k c c e e1 f1 f l
5S rDNA
g
e2
f2
a b c' d b c d e e1 f
M2A
pArt 5H-b
h j
i k c c e e'
5S rDNA
g'
f
pArt 5-b

inverted repeat (TTTGT/ACAAA), flanked by a short direct repeat, TTAA, suggesting a transposition event.

Fig. 7 shows the sequences of the 3' end of the histone genes for pArt5H-a and pArt5H-b. The region contains the coding sequence for the last 32 aminoacids (pArt5H-a) or 27 aminoacids (pArt5H-b) of histone H2A. The sequences are identical to the consensus (22) except for the Gly-98 (Ser in *Artemia*) and the last six aminoacids, very variable among different species. The conserved region (of 23 bp), found 40 bp after the termination codon, has 83% similarity with the consensus (the four changes found do not affect the 16 bp internal hairpin structure of this region). The purine-rich motif, RAAAGA, is found 8 bp after the conserved region, although in pArt5H-a it has a G to T transversion. This motif is found again after 80 bp, the distance needed for correct termination of transcription (23). In summary, all the elements described for correct termination and messenger stability are found (23-25). The finding of H2A as the closest histone to 5S rRNA is in contrast with the data of Andrews *et al.* (8), who suggested that the 5S rRNA gene was located between H2B and H1, although based only in hybridization with heterologous probes.

Based on the similarity of the sequences surrounding the 5S rRNA coding sequence, we think that the 5S rRNA gene contains, besides the 120 bp of coding sequence, region "g" upstream and regions "h", "i" and "j" downstream, which give a total of 315 bp. The 5S rRNA coding sequence in the three recombinants is very similar; in pArt5H-a it is identical to the sequence described for 5S rRNA from the San Francisco Bay population by Diels *et al.* (26) by RNA sequencing. pArt5H-b has a T to C change in position 118 and pArt5-b has three changes: T to C (position 17), C to A (position 54) and T to C (position 111).

Recently, it has been shown that transcription by RNA polymerase III is not only dependent of the internal control regions but also of 5' promoter elements (27-30). These regions seem to be located at approximately -30 and -60 bp from the transcription initiation site, and have been identified by mutation and deletion analysis. The region at -30 is a "TATA-like" motif, not very well defined (31). The -60 region is pyrimidine-rich, and has been studied in U6 snRNA genes from mouse (32) and from *X. tropicalis* (33). Similar sequences are found in *Artemia* 5S rRNA gene in pArt5H-a at position -41 to -56 (711 to 725 in Fig. 5) or at position -43 to -56 (804 to 817 in Fig. 5) in pArt5H-b.

The 3' regions "h" and "i" are T-rich sequences acting as termination signals for RNA polymerase III transcription (34).

Fig. 6. Schematic representation of the sequences shown in Fig. 5. Letters and symbols are the same as in Fig. 5. Small dashes above regions 'a', represent the RAAAGA motif discussed in the text.

```
            Lys Leu Leu Gly Gly Val Thr Ile Ala Gln Gly Gly Val Leu Pro Asn Ile Gln
                        Ser                                                        112
pArt5H-a    AAG CTT CTG TCG GGG GTC ACC ATT GCC CAA GGA GGC GTT TTG CCC AAT ATT CAA   54
pArt5H-b                  . ..T ... ... ... ... ... ... ... ... ... ..C ... ...    40

            Ala Val Leu Leu Pro Lys Lys Thr Glu Ser His His Lys Ala Lys Gly Lys
                                                Lys Pro Ala                        126
pArt5H-a    GCA GTC CTT CTA CCA AAG AAG ACT GAA AAA CCG GCA AAG GCT TAA             99
pArt5H-b    ..C ... ... ... ... ... ... ... ... ... G.. ... ... ... ...            85
                                                    Ala

                                                          G       C   C C
pArt5H-a    ATGAATCTAATTTTTAGCTCCAGGCCCAACCCCCAAATTAACCAACAGCCCTTTTAAGGGCTACAAA    166
pArt5H-b    ..A.T.....C-...G.AC.T......T.....-.............................        150

              AGA
pArt5H-a    TTAATTGAATTGCTCTATAGCATGTGACCATCTGGAAAGGACAAAGCTATGAAATTTAGGATACTGA    233
pArt5H-b    ..........GA.A.............CG...G....G.........A.....A....A.....       217

              A
pArt5H-a    ATGT--GAAAAGGAAAA                                                      248
pArt5H-b    .C..AA.......GGGCGTGGGGGG                                              242
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Fig. 7. 3' region of the H2A sequence from plasmids pArt5H-a and pArt5H-b. The underlined sequences are the consensus ones (21, 22). Points in the nucleotide sequences represent identical bases. Dashes represent deleted bases to maximize homology. Only aminoacid changes are shown. Numbers in the aminoacid sequence correspond to the consensus sequence; numbers in the nucleotide sequences refer to Fig. 5.

DISCUSSION

We have studied the genomic organization of the 5S rRNA-histone repeat in different populations of *Artemia*. All populations studied have 5S rRNA and histone genes linked in one unit, and in all cases there are two types of units in every population. These two types are of 9.0 and 8.5 kb in American bisexuals, and of approximately 12 kb in parthenogenetic populations from Eurasia. The clones described by Andrews *et al.* (8) must be of the type we have called before type II from American bisexuals, as these are the only ones that have *Eco*RI sites, and their genotheque was made in lCharon4A phages (7). Further support for this hypothesis comes from the diagram of the upper part of Fig. 6, that corresponds to the data of these authors (9). The only common region with the clones described in this work is the 5S rRNA gene alone, although with minor differences in the arrangement of the regions "h", "i" and "j". Upstream of region "g" the sequence has no similarity with our type I repeat. We do not know, however, if there is the same kind of organization of type I repeats in the type II repeats closer to the histone genes.

pArt5H-b is of parthenogenetic origin. As the experiment shown in Fig. 3 demonstrate, these repeats are of approximately 12 kb; therefore, pArt5H-b must be a 9 kb *Sal*I fragment of this type of repeats.

Artemia 5S rRNA-histone genes have very large spacers, in contrast with other species, from mammals to lower eukaryotes. We do not know whether there are other coding sequences in this region; hybridization experiments, however, demonstrate that these spacers are specific for a given population. For instance, the 1.0 kb *BglI*-*PstI* fragment of pArt5H-a only hybridizes with American populations, and the 3 kb *PvuII*-*SalI* fragment of pArt5H-b hybridizes with populations from Eurasia, both bisexuals and parthenogenetics (data not shown). This is in agreement with our previous findings about the distribution of satellite I (18) and rRNA (Medina, R. *et al.* in preparation), that suggest that the appearance of parthenogenesis in the old world *Artemia* occurred later than the geographical isolation of *Artemia* between the new and the old worlds.

Our sequencing results clearly demonstrate that the histone gene that is closer to the 5S rRNA genes is H2A. This is in contrast to the data of Andrews *et al.* (8), that reported that the 5S rRNA gene is between H2B and H1. The H2A gene studied, and presumably the whole histone cluster, belongs to the histone genes expressed during the S phase, and whose mRNAs are not polyadenylated (35). It is surprising, then, that region "c" contains the canonical polyadenylation signal, AATAAA. In pArt5H-a, there is only one signal, located 262 bp from the termination codon. In pArt5H-b there are two "c" regions after H2A and another two after the 5S rRNA gene. Although Alterman *et al.* (36) have found in mouse cell hybrids histone mRNAs that contain the hairpin structure and are correctly polyadenylated, we do not think that these signals are functional in these genes in *Artemia*, as its genome is very A-T rich (18) and therefore the sequence AATAAA is likely to be found by chance in a non-transcribed region.

The linkage between 5S rRNA and histone genes posses the problem of its origin. We think that the 5S rRNA gene (315 bp) seems to have invaded the histone repeat, as the same type of organization of small repeated regions is seen at both sides of the 5S rRNA gene and in repeats coming from different *Artemia* populations. This invasion could have take place by transposition; in pArt5H-a, the 5S rRNA gene is bounded by the direct repeat AACAAA (positions 622 and 948 in Fig. 5); the same repeat is found in the sequence of Bagshaw *et al.* (9). Parthenogenetic organisms have a different repeat, AAATAAAC (positions 731 and 1033 in pArt5H-b, Fig. 5).

The linkage between 5S rRNA and other transcriptional units is well known in prokaryotes and (as mentioned in the Introduction) in protists and fungi (3), where the 5S rRNA gene is linked to the 18S-5.8S-28S unit. Pace *et al.* (37) have suggested that the lack of linkage between the 5S rRNA gene and the large rRNAs genes would be a primitive characteristic of all eukaryotes. If so, then, the linkage between the 5S rRNA gene and other transcriptional units should be a later event in evolution.

There is no reason *a priori* to restrict the transposition of 5S rRNA genes only to the other rRNA genes. The mechanisms by which this process took place should fall in what Dover (38) describes as molecular drive; it would be difficult to imagine this transposition event before amplification of histone and 5S rRNA genes. Molecular drive, on the other hand, could homogenize the 5S rRNA-histone repeats and make disappear the isolated repeats.

Another important question is whether this linkage is particular to *Artemia* or it is a more general phenomenon. Insects, another class of the phylum Arthropods do not have the linkage (2). In the class Crustacea there is very little information. Drouin *et al.* (4) have found that in the genus *Calanus* (subclass Copepoda) 5S rRNA genes are again linked to the large rRNA repeats. We have preliminary evidence that shows that in lobster and crayfish (subclass Malacostraca, order Decapoda), 5S rRNA and histone genes are not linked (data not shown). Clearly, more work is needed with other primitive crustaceans and other arthropods, like kelycerates, or even lower species in the phylogenetic tree, like annelids, to analyze the distribution of this unusual gene linkage.

ACKNOWLEDGEMENTS

We thank helpful suggestions and discussions of other members of our laboratory and the technical help of Elvira Domínguez. This investigation was supported by grants from the Comisión Asesora para la Investigación Científica y Técnica (BT/18) and Fondo de Investigaciones Sanitarias de la Seguridad Social (86/715).

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This paper is dedicated to the memory of our friend Beatriz Batuecas.

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