DNA REASSOCIATION KINETIC ANALYSIS OF THE BRINE SHRIMP, ARTEMIA SALINA

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SUMMARY. DNA reassociation kinetics have been partly elucidated for the brine shrimp Artemia salina, using calf thymus DNA as a standard. The Artemia single-copy DNA sequences comprise 45% of the genome; sequences having a repetition frequency of about 2-90 are not detectable. The average repetition frequency of the intermediately redundant DNA component is about 5,000 copies. Reassociation kinetic data are consistent with a unit genome size of 1.5 pg.

Encysted blastula and/or gastrula stage embryos of the brine shrimp Artemia salina provide a convenient biological system for biochemical studies. The dehydrated cysts are commercially available in bulk, and will resume development when immersed in artificial sea water. Following a series of cellular reorganizations within the cyst, nauplius larvae hatch about 24 hours after the onset of incubation. Following hatching, larvae undergo marked changes in the rate of RNA synthesis (18) and in the relative levels of activity of RNA polymerases I and II (4)... of interest in relation to mechanisms of transcriptional regulation. This system is also useful as a model for translational control, as the encysted embryo contains a storage form of mRNA which fails to associate with ribosomes until an advanced stage in embryonic development (11, 12, 22). This is thought to be analogous to the storage of inactive maternal mRNA in sea urchin eggs, which becomes translated only after fertilization (13, 30).

Chromosomal DNA sequence organization has been hypothesized to play an important role in eucaryotic gene regulation at the transcriptional level (6, 10). During a study of DNA sequence organization in the brine shrimp, the reassociation kinetics for this species were determined. It is anticipated that

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these results—which are the subject of this report—should now facilitate certain analyses of RNA components during embryonic development in this species.

MATERIALS AND METHODS

Dehydrated brine shrimp embryos were obtained from the Metaframe Corp., Newark, Calif. These shrimp have their origin in San Francisco Bay, Calif. The cysts were hatched in artificial sea water in 7 g batches at room temperature in a 5 gallon aquarium with vigorous aeration. After 48 hours, the aeration was discontinued and the aquarium was masked to exclude light from all but one end—which was illuminated with a lamp. Swimming larvae congregated at the lighted end of the aquarium, whereas egg capsules and unhatched cysts either remained on the bottom or floated at the surface. The swimming larvae were siphoned, taking care to avoid egg capsules, and collected by filtration. After thoroughly washing the shrimp with fresh artificial sea water, DNA was immediately isolated.

DNA isolation. Brine shrimp or fresh calf thymus were homogenized in a stainless steel Waring vessel in 70 ml of ice-cold 0.10 M NaCl- 0.20 M disodium EDTA, pH 8.0. The detailed subsequent DNA isolation steps have been previously described (26). Each 7 g batch of cysts ultimately yielded about 8.5 mg of highly-purified DNA, having an A_{260}/A_{230} ratio of about 2.2. The quality of this DNA was further established by characterization of its thermal denaturation properties.

Thermal denaturation of DNA. DNA was dialyzed to 0.015 M NaCl- 0.0015 M sodium citrate, pH 7.0. Thermal denaturation profiles on DNAs having an initial concentration of about 0.7 O.D.260 units were automatically recorded in a Gilford spectrophotometer equipped with a thermal programmer set to provide a linear temperature increase of 1°C/minute. As a control, calf thymus DNA was simultaneously melted in the same thermal block.

The T_m (temperature at which DNA is 50% denatured) of each sample was accurately determined by replotting the data on normal probability paper (14). The %(G+C) for each sample was calculated from this T_m value, using the relationship derived by Mandel and Marmur (17):

$$\%(G+C) = 2.44(T_m - 53.9)$$

Kinetics of DNA renaturation. Our procedures for following the renaturation of short single-stranded DNA fragments have been published in detail elsewhere (26). We have previously shown that sonication of long DNA molecules yields a population of fragments having a length frequency distribution of about 165-330 nucleotides, as determined by electron microscopy. It is important that the fragment length be short, as longer fragments would be expected to contain a significant proportion of not only repeated-copy but also single-copy sequences, which would result in an over-estimate of the proportion of repeated-copy material when the reaction mixture is assayed by hydroxyapatite chromatography. This is owing to the widespread genome organizational pattern (10) consisting of alternation of middle repetitive sequences about 300 nucleotides long with non-repetitive sequences about 800-1200 nucleotides long.

RESULTS AND DISCUSSION

Thermal denaturation. A prominent dAT satellite component comprises a significant fraction of many crustacean genomes (25, 23, 5, 27). The Artemia

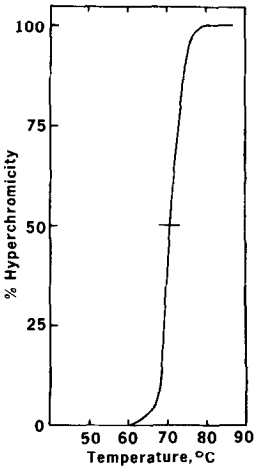

Fig. 1. Thermal denaturation profile of Artemia DNA in 0.015 M NaCl-0.0015 M sodium citrate, pH 7.0. The T_m (bar) is at 70.7°C, reflecting a %(G+C) content of 41.0%.

genome melts smoothly with no obvious indication of prominent satellite components, as shown in Figure 1. However, minor components of 1-2% would not be detected by this approach. When these data are re-plotted on normal probability paper to more accurately assess the T_m, Artemia DNA is found to have a T_m of 70.7°C. This value predicts a %(G+C) content of 41.0% (17), in good agreement with the 40% value obtained for this species by other workers (1). Confidence in these values is given by the fact that bovine DNA, run as a control, has a T_m of 70.9°C, which predicts a 41.5% (G+C) content. This compares with a literature value of 42.0% (24).

Good quality high molecular weight DNA having about 40% (G+C) content should give an absorbance increase at 260 nm of about 40% upon complete thermal denaturation (17). The Artemia DNA yielded a 40.0% absorbance increase, which reflects a high degree of purity and minimal degradation.

Renaturation kinetics. The results of our renaturation analysis are given in Figure 2. Calf thymus DNA was run as a standard, since its renaturation kinetics are well characterized (7, 8). The slowest reassociating Artemia sequences comprise 45% of the genome, and have an uncorrected $cot\frac{1}{2}$ of 1900.

Table 1. Comparison between kinetic complexity and haploid DNA content

DNA species	% G+C	% unique	$Cot_{\frac{1}{2}}$ (mole-sec/liter)		Haploid genome (pg)
			Corrected for repeated sequences	Corrected to 50% G+C	
B. taurus (standard)	42	58	3,900	3,120	3.2[a]
A. salina	41	45	1,900	1,460	1.5[b]

[a] References #16, 21 and 28.

[b] Calculated from kinetics.

This value was determined by fitting a calculated theoretical second-order kinetic curve (29) to the observed data points (26).

Since the data points show no consistent deviation from single component second-order reaction kinetics above cot 30, we interpret this to mean that repetition frequencies from about 2-90 are rare for this species. This result is very similar to that obtained for two other crustacean species (crabs) which we earlier examined (26), and two additional crab species studied by Christie et al. (9). In the latter two studies, it was also found that a distinct plateau separates the single-copy from the repeated-copy components in the cot kinetic plots. Accordingly, we suspect that the slowest reassociating component of the Artemia curve also represents single-copy sequences.

If the slowest reassociating Artemia sequences do represent the single-copy component of the genome, then comparison of the single-copy bovine kinetics to those for Artemia, after appropriate corrections for repeated sequences (15) and differences in %(G+C) content (29) should give a close estimate of the Artemia haploid genome size. These calculations, entered in Table 1, predict an Artemia haploid genome size of 1.5 pg. The only published Artemia haploid DNA value we know is 3.0 pg (20)... almost precisely twice

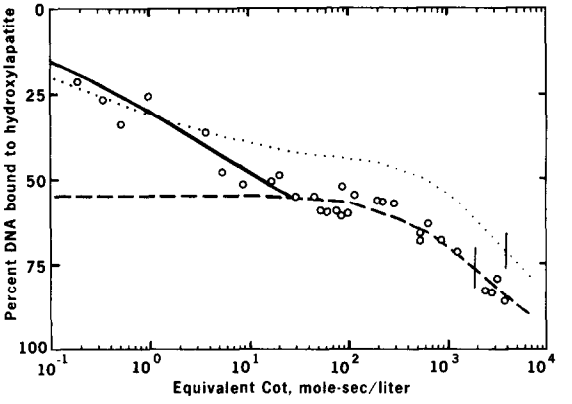

Fig. 2. Renaturation kinetics assayed by hydroxylapatite chromatography for B. taurus (the standard) is shown by dotted line; A. salina is shown by open circles. The dashed line represents single component theoretical second-order reaction kinetics which best fit the observed data points. The A. salina genome at the 60°C in 0.12 M sodium phosphate buffer criterion contains 45% single-copy DNA sequences (dashed line) which by themselves have an apparent $cot\frac{1}{2}$ (bar) of 1,900. The bovine genome contains 58% single-copy DNA sequences which by themselves have an apparent $cot\frac{1}{2}$ (bar) of 3,900.

the value predicted from our kinetic data. Since the 3 pg value was obtained by Feulgen microspectrophotometry of individual nuclei from entire organism homogenates (then divided by two to yield a haploid value), rather than directly from gametes, it is possible that polyploid somatic nuclei were measured. Polyploidy is known to be widespread in a number of crustacean species (3). Indeed, polyploid races of Artemia have been described (2), and the 3 pg value could have been recorded from such a race. Polyploidy would not be expected to change the relative concentration of any DNA sequences involved, so that the DNA renaturation kinetic data should be identical (19) for polyploids (haploid value: 6/2 pg) and diploids (haploid value: 3/2 pg).

Kinetic data yield the unit genome size, defined (15) as "the chromosomal DNA of a cell such that the least frequent nucleotide sequences are present only once, and such that all nucleotide sequences are represented in their original proportions." We find the unit genome size for A. salina to be 1.5 pg. The potential informational complexity can not be greater than this value for this species.

Table 2. DNA fractions resolvable by reassociation kinetics

DNA species	Percent of nuclear DNA	Multiplicity frequency range	Average multiplicity frequency
Artemia salina	45	1	1
	rare	2-90	
	39	90-20,000	5,000
	16	>20,000 copies	
Libinia emarginata[a]	30	1	1
	rare	2-100	
	40	100-30,000	3,000
	30	>30,000 copies	
Cancer borealis[a]	30	1	1
	rare	2-200	
	30	200-30,000	2,500
	40	>30,000 copies	
Gecarcinus lateralis[b] & *Geryon quinquedens*[b]	30	1	1
	rare	10-250	
	25	1,200-12,000	not stated
	25-30	>12,000 copies	

[a] Recalculated from Vaughn (26).

[b] From Christie et al. (9).

The sequences renaturing below about cot 30 (Figure 2) represent the intermediately repetitive component of the *Artemia* genome. The average multiplicity frequency for this DNA class is no more than 5000: comparable to values obtained for this class from other crustacean species (26, 9). We have only carried our kinetic analysis to about cot 2×10^{-1}. About 20% of the *Artemia* genome consists of very rapidly renaturing sequences (>10,000 copies each) which have already renatured at this cot value. For purpose of comparison, available crustacean DNA reassociation kinetic data from all sources has been tabulated in Table 2. It can be readily seen that a good deal of similarity exists amongst these diverse species... of which four are relatively highly evolved crabs and one (*Artemia*) is a descendant of a primitive line of crustacea.

Knowledge of the reassociation kinetics of *Artemia* chromosomal DNA should facilitate certain analyses of RNA components during embryonic development in this species. In addition, this information will be valuable for the subsequent establishment of the DNA sequence organization pattern.

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