Comparative DNA Renaturation Kinetics in Amphibians

Neil A. Straus

PNAS 1971;68;799-802
doi:10.1073/pnas.68.4.799

Proc. Nat. Acad. Sci. USA
Vol. 68, No. 4, pp. 799–802, April 1971

Comparative DNA Renaturation Kinetics in Amphibians

NEIL A. STRAUS*

Department of Botany, University of Toronto, Toronto, Ontario, Canada

Communicated by Richard B. Roberts, January 11, 1971

ABSTRACT **Amphibian haploid genome sizes vary from 9×10^8 to 8×10^{10} nucleotide pairs. The rate of reassociation of DNA from amphibians of different genome sizes has been employed to eliminate one of the theoretical models of chromosome structure.**

***Scaphiopus couchi*, *Bufo marinus*, and *Rana clamitans*, whose haploid genome sizes are in the ratio 2:7:10, all contain sequences of DNA represented once in the haploid genome. This indicates that their chromosomes are not composed of identical lateral strands (polynemy). The relative frequencies of repetition of DNA sequences are different for the various species of amphibians.**

The observed frequencies of repetitive DNA sequences in amphibians do not show the relationships expected if amphibians form a polyneme series.

Autoradiography and electron microscopy have shown that the chromosomes of *Escherichia coli* (1), P_{22} (2), and T_2 (3) are single Watson–Crick helices. Greater DNA content per cell and the association of protein with DNA hamper investigation into the structure of eukaryotic chromosomes. Phenomena such as large polytene chromosomes of diptera that can exhibit differential replication during their formation (4), somatic amplification of ribosomal cistrons in oocytes (5, 6), and incorporation of viral DNA into mammalian chromosomes (7) further complicate resolution of eukaryotic chromosome structure.

Models of chromosome structure of higher organisms must account for the high frequencies of DNA sequence repetition demonstrated by DNA renaturation kinetics (8) and DNA–RNA hybridization (9, 5). The argument that these sequences result from somatic amplification seems improbable in view of the evidence of Swift (10) that almost all the nuclei of somatic tissues contained twice as much DNA as spermatid nuclei.

Differential lateral multistrandedness in chromosomes has at times been used to account for large changes in DNA content per nucleus within certain taxonomic groups that do not have corresponding changes in chromosome number (11, 12). Differential polynemy is a model for chromosome structure that postulates the existence of many lateral identical strands of DNA double helix.

Amphibians exhibit a wide range in genome size (13). If amphibians form a classical polyneme series, the DNA renaturation kinetics of all the members of the group should be identical, since the addition of identical lateral strands does not alter the relative frequency of any DNA sequence. "Unique" DNA can be present only in the lowest member of a polyneme series.

* Present address: Department of Terrestrial Magnetism, Carnegie Institution of Washington, Washington, D.C. 20015.

MATERIALS AND METHODS

Animals

The animals used were *Scaphiopus couchi* (The Pet Corral, Arizona), *Bufo marinus* and *Ambystoma tigrinum* (The Lemberger Co., Wisconsin), *Necturus maculosus* (Boreal Biological Laboratories, Ontario), and *Rana clamitans* (collected north of Toronto, Ontario).

DNA isolation

DNA was isolated from testes and livers of *R. clamitans* and *S. couchi* by a combination of the methods of Marmur (14) and Berns and Thomas (15), and from blood of *B. marinus*, *A. tigrinum*, and *N. maculosus* by a slight modification of the method of Miyagi *et al.* (16). The detailed methods of isolation are reported elsewhere (17).

DNA shearing

All samples of DNA, in solutions between 0.1 and 3 × SSC, where SSC = 0.15 M NaCl–0.015 M sodium citrate, were forced through a needle valve by pressure generated from an Aminco pump (no. 45-13715) driven by compressed air at 90 psi (6 atm). The pressure drop over the valve is rated at 40,000 psi. The DNA solution was collected dropwise and filtered through metricel GA-6 filter (0.45-μm-pore diameter). These filters do not bind DNA (18). Differences in salt concentrations of the solutions in which the DNA was sheared have no significant effect on rate, as judged by the rates of renaturation of DNA from T_4 sheared in 0.1 × SSC and 5 × SSC. Rates of renaturation of amphibian DNA were compared with that of *E. coli* sheared at 50,000 psi. This comparison is legitimate since the rate of renaturation is proportional to the square root of the fragment size (19) and the sizes of fragments of DNA sheared at 12,000 psi and 50,000 psi differ only by a factor of 2 (20).

DNA renaturation

Sheared samples of DNA dissolved in 0.12 M PB (0.06 M monobasic sodium phosphate–0.06 M dibasic sodium phosphate) were heated to 100°C for 3 min and then rapidly cooled to and incubated at 60°C. After incubation, single-stranded DNA was separated from double-stranded DNA by passage of the solution over hydroxyapatite (Calbiochem, boiled and washed with 0.12 M PB before use) in a water-jacketed column (20). Single-stranded DNA passes through the column and the double-stranded DNA, which is adsorbed under these conditions, was eluted with 0.12 M PB buffer at 100°C. For long incubations, minute amounts of ^{32}P-labeled, sheared *E. coli* DNA, generously provided by D. Kohne and D. Canter

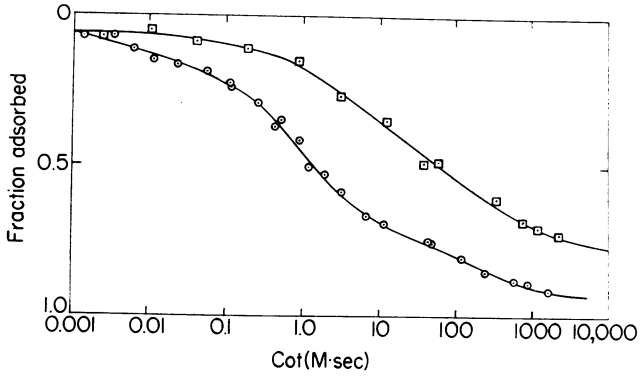

FIG. 1. Renaturation of sheared *Ambystoma tigrinum* DNA ⊡ (A_{260} = 0.074–30.0) and *Necturus maculosus* DNA ⊙ (A_{260} = 0.116–23.2). Fraction adsorbed refers to the fraction of DNA that has renatured (double-stranded DNA adsorbs to hydroxyapatite). The solid lines are computer-plotted least-square fits of three components to the data.

of the Carnegie Institution of Washington, were added as a check on whether the incubation mixture was suffering from thermal degradation. Radioactivity was monitored by Cerenkov counting of ^{32}P in the tritium channel of a Packard scintillation counter (21).

Renaturation plots

The rate of renaturation is inversely proportional to the genome size for DNA sequences represented only once in the genome. Repeated sequences reassociated more rapidly than single-copy sequences.

The fraction of DNA that has renatured was plotted logarithmically against Cot (the product of DNA concentration and the time of incubation). $(Cot)_{1/2}$ is the Cot value of a component when it is half-reassociated. The $(Cot)_{1/2}$ of a component is the inverse of its rate constant of reassociation. The Cot values—in moles per liter times seconds—are given approximately by the product of half the absorbance at 260 nm of the DNA in solution and the time of incubation in hours (8).

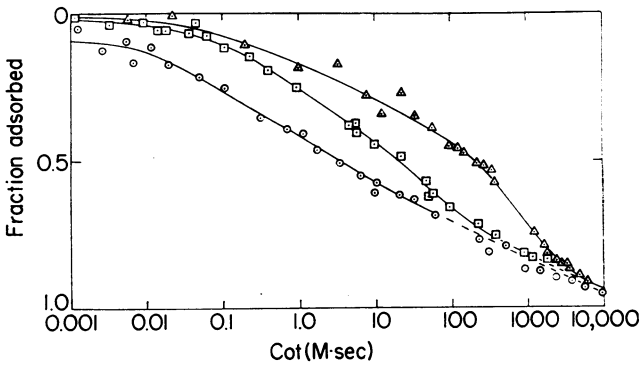

FIG. 2. Renaturation of sheared *Scaphiopus couchi* DNA △ (A_{260} = 0.417–124), *Rana clamitans* DNA ⊡ (A_{260} = 0.085–116), and *Bufo marinus* DNA ⊙ (A_{260} = 0.103–157). The solid lines are computer-plotted least-square fits of three components to the data. The broken lines add the theoretical unique components whose existence is demonstrated in Figs. 5 and 6.

TABLE 1. *Amphibian haploid genome sizes*

Species	Relative size of genome (ref. 13)	Nucleotide pairs ($\times 10^9$) of DNA per haploid nucleus (calcd)	Expected $(Cot)_{1/2}$ (M·sec) (calcd)
Scaphiopus couchi	0.16	0.9	580
Bufo marinus	0.69	3.8	2,500
Rana pipiens	1.00	5.6	3,600
Rana clamitans	1.07	6.0	3,900
Ambystoma tigrinum	5.64	32.0	20,500
Necturus maculosus	13.5	76.0	49,000

The haploid genome of *R. pipiens* contains about 5.8 pg of DNA (13, 22) and hence 5.6×10^9 nucleotide pairs. Thus, the genome of *R. pipiens* is about 1240 times as large as that of *E. coli* (4.5×10^6 nucleotide pairs, ref. 23). Since the $(Cot)_{1/2}$ of *E. coli* DNA—incubated in small amounts in concentrated solutions of amphibian DNA—was equal to 2.9 ± 0.5 M·sec, the $(Cot)_{1/2}$ of unique *R. pipiens* DNA is expected to be 3600 (1240 × 2.9) M·sec. Table 1 contains the genome sizes of amphibians relative to that of *R. pipiens* and the expected $(Cot)_{1/2}$ values of the single-copy DNA in these amphibians.

RESULTS

Fig. 1 shows the reassociation plots of DNA from the urodeles *N. maculosus* and *A. tigrinum*, and Fig. 2 those for the anurans *S. couchi*, *B. marinus*, and *R. clamitans*.

The data of *S. couchi* were computer-fitted by a least-squares analysis of three components (Table 2). The last component had a $(Cot)_{1/2}$ value of 770 and represents 60.4% of the genome. Since the theoretical $(Cot)_{1/2}$ for the single-copy DNA of *S. couchi* is expected to be 580 (Table 1), it is assumed that the last reacting sequence of *S. couchi* is represented once in the haploid genome.

In contrast, the genomes of the other amphibians studied are composed mostly of repetitive DNA. Because the last

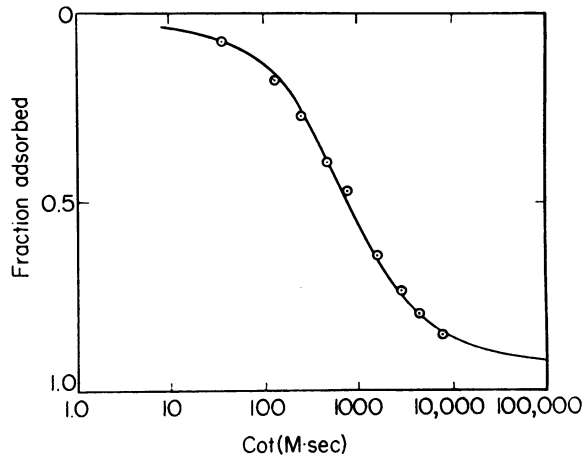

FIG. 3. Cot plot for a portion of *B. marinus* DNA isolated as unrenatured DNA after incubation to Cot = 450 (A_{260} = 69.1). The solid line is a least-square fit of a single component to the data. The $(Cot)_{1/2}$ (corrected for the fact that 10% of the absorbance is nonreactive material) is 660 M·sec.

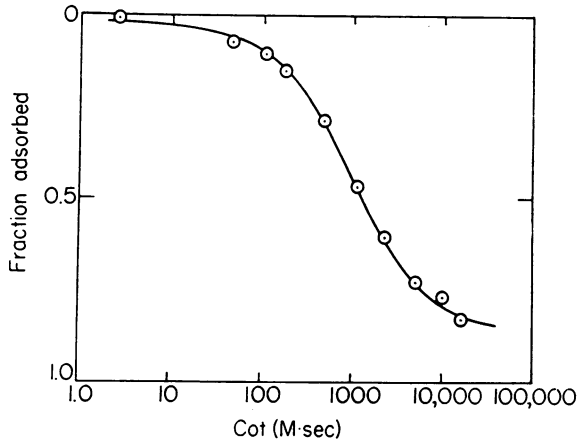

FIG. 4. Cot plot for a portion of *R. clamitans* DNA isolated as unrenatured DNA after incubation to Cot 540 (A_{260} = 106). The solid line is a least-square fit of a single component to the data. The $(Cot)_{1/2}$ is 850 M·sec (corrected for nonreactive material).

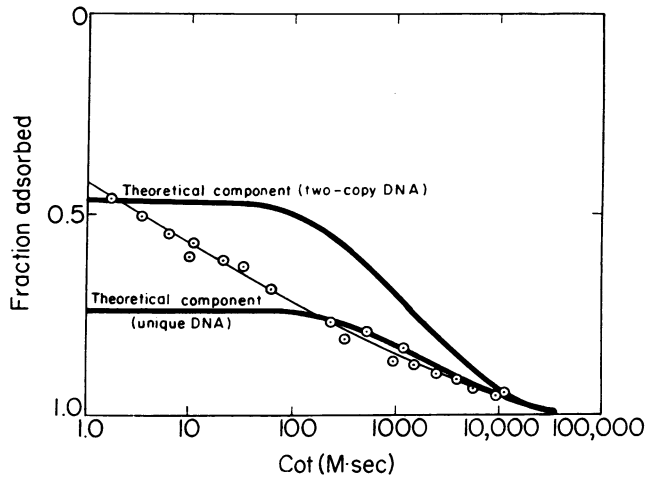

FIG. 5. Cot plot of *B. marinus* (whole genome) with the plots of the last component drawn in on the assumption that the last component to renature is either two-copy DNA (*above*) or unique DNA (*below*).

fraction to renature in *B. marinus* and *R. clamitans* is a minor part of the total genome, a large amount of this fraction was isolated as the DNA that remained unrenatured after Cot values of 450 and 540 M·sec, respectively. The unreassociated DNA was concentrated by repeated lyophilization and dialysis. Figs. 3 and 4 contain the Cot plots of the fractionated DNAs of *B. marinus* and *R. clamitans*, respectively. Least-square fits of the theoretical curves to these Cot plots showed that the fractions renatured with the kinetics of single components whose $(Cot)_{1/2}$ values are 660 (*B. marinus*) and 850 (*R. clamitans*).

For an isolated portion of DNA with one repetition frequency, $(Cot)_{1/2}$ of the isolated component equals β times the $(Cot)_{1/2}$ of the component when this is determined as part of total DNA renaturation, where β is the fraction of the genome that forms the component (24). The expected $(Cot)_{1/2}$ values of unique and two-copy DNA in *B. marinus* are 2500 (Table 1) and 1250, respectively. Since the $(Cot)_{1/2}$ value of the isolated sequence is 660, unique DNA should constitute 26% of the whole genome and two-copy DNA should be 53% of the whole genome. Corresponding values for *R. clamitans* are 22 and 44%. Fig. 5 contains the Cot plot of the whole genome of *B. marinus*, on which are superimposed the theoretical Cot plots of the last component, assuming either that the last component to renature is unique or that it is two-copy DNA. Fig. 6 is a similar plot for *R. clamitans*. For both *B. marinus* and *R. clamitans* the theoretical unique component is the right size, but two-copy DNA is too large to represent the last renaturing components. Therefore, the last sequences to renature in *B. marinus* and *R. clamitans* are uniquely represented in the haploid genomes of these animals.

Three components were fitted (by a least-square analysis) to all the "nonunique" portions of the amphibian plots in Figs. 1 and 2 by means of a computer program designed by R. Britten. Two components were sufficient to fit a curve to the "nonunique" portion of the Cot plot of *S. couchi*. Table 2 contains the results of this analysis, from which two general features arise. First, all the genomes studied contain different fractions of DNA at different frequencies of repetition; second, the animals of larger genome size (see Table 1) generally have higher frequencies of repetitions in their DNA sequences than those of smaller genome sizes.

CONCLUSION

Single-copy DNA has been shown to exist in certain mammals (8, 20) and *Xenopus* (25). The present work indicates that unique DNA is also present in *S. couchi*, *B. marinus*, and *R. clamitans*. Since unique DNA can only be present in the lowest member of a classical polyneme series, amphibians cannot form a classical polyneme series. The existence of single-copy

TABLE 2. *Frequency distributions of DNA sequences in amphibians, estimated from DNA renaturation rates*

Species	Fastest-renaturing component			Moderate component			Slow component			"Unique" component			Theoretical $(Cot)_{1/2}$ of unique DNA (M·sec)
	Fraction of genome	$(Cot)_{1/2}$ (M·sec)	Repet. freq.	Fraction of genome	$(Cot)_{1/2}$ (M·sec)	Repet. freq.	Fraction of genome	$(Cot)_{1/2}$ (M·sec)	Repet. freq.	Fraction of genome	$(Cot)_{1/2}$ (M·sec)	Repet. freq.	
S. couchi	3.5	0.108	5,360	23.5	5.8	100	...	...	...	60.4	770	1	580
B. marinus	26.0	0.0518	48,200	27.4	2.47	1,000	14.3	72.4	35	26	2540	1	2,500
R. clamitans	9.1	0.0544	71,700	26.9	0.955	4,080	40.2	41.8	93	22	3860	1	3,900
A. tigrinum	6.1	0.0083	2.5×10^6	36.9	5.88	3,480	27.0	379	54.1	?			20,500
N. maculosus	13.6	0.0073	6.7×10^6	54.1	0.984	49,800	20.2	147	334	?			49,000

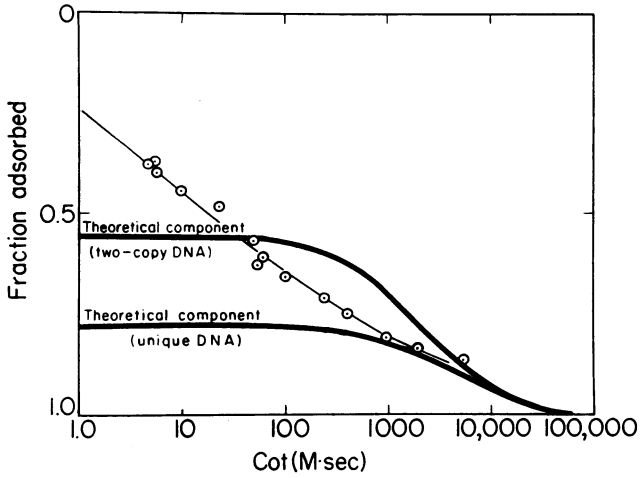

FIG. 6. The Cot plot for *R. clamitans* (whole genome) with the plots of the last component drawn in on the assumption that the last component to renature is either two-copy DNA or unique DNA.

DNA could be cited as evidence for mononemic chromosomes if certain assumptions are made about chromosome structure.

Since polynemy does not change the basic concentration (per unit of DNA) of any genetic sequence in the genes involved, the DNA renaturation kinetics of all the animals forming a polynemic series should be identical. The great differences among the renaturation kinetics of the amphibians studied again indicate that they cannot form a classical polynemic series.

The renaturation kinetics indicate that the differences in genome size are accompanied by large differences in the distribution of repetition frequencies of DNA sequences (Table 2). Animals of larger genome size generally have higher frequencies of sequence repetition than animals with smaller genomes. The larger genomes therefore appear to have been constructed, at least in part, by repeating small fractions of the genomes a large number of times.

I thank Dr. K. H. Rothels, who as a patient and helpful thesis advisor made this work possible; and I acknowledge the encouragement and helpful advice of Drs. R. J. Britten, D. E. Kohne, and M. Miyagi for their encouragement and helpful advice, and Drs. B. H. Hoyer and R. B. Roberts for improving the manuscript. This work was supported by grants from the National Cancer Institute of Canada, and the Medical Research Council of Canada. I am grateful to the Carnegie Institution of Washington, Department of Terrestrial Magnetism for the use of their facilities.

1. Cairns, J., *J. Mol. Biol.*, **6**, 208 (1963).
2. Rhoades, M., L. A. MacHattie, and C. A. Thomas, Jr., *J. Mol. Biol.*, **37**, 21 (1968).
3. Thomas, C. A., Jr., and L. A. MacHattie, *Proc. Nat. Acad. Sci. USA*, **52**, 1297 (1964).
4. Rudkin, G. T., *Genetics*, Suppl. 1, Part 2, **61**, 227 (1969).
5. Brown, D. C., and I. B. Dawid, *Science*, **160**, 272 (1968).
6. Gall, J. G., *Genetics*, Suppl. 1, Part 2, **61**, 121 (1969).
7. Dulbecco, R., *Ann. Intern. Med.*, **70**, 1019 (1969).
8. Britten, R. J., and D. E. Kohne, *Science*, **161**, 529 (1968).
9. Ritossa, F. M., and S. Spiegelman, *Proc. Nat. Acad. Sci. USA*, **53**, 737 (1965).
10. Swift, H. H., *Physiol. Zool.*, **23**, 169 (1950).
11. Rothfels, K., and M. Heimburger, *Chromosoma* (Berlin), **25**, 96 (1968).
12. Rothfels, K., E. Sexsmith, M. Heimburger, and M. O. Krause, *Chromosoma* (Berlin), **20**, 54 (1966).
13. Sexsmith, E., *DNA Values and Karyotypes of Amphibia*, PhD thesis, University of Toronto, 1968.
14. Marmur, J., *J. Mol. Biol.*, **3**, 208 (1961).
15. Berns, K. I., and C. A. Thomas, Jr., *J. Mol. Biol.*, **11**, 476 (1965).
16. Miyagi, M., D. Kohl, and R. A. Flickinger, *J. Exp. Zool.*, **165**, 147 (1967).
17. Straus, N. A., *The Renaturation Kinetics of DNA from Amphibians of Differing Genome Sizes*, PhD thesis, University of Toronto, 1970.
18. Kohne, D. E., *Biophys. J.*, **8**, 1104 (1968).
19. Wetmur, J. G., and N. Davidson, *J. Mol. Biol.*, **31**, 349 (1968).
20. Britten, R. J., and D. E. Kohne, *Carnegie Inst. Washington, Yearbook*, **65**, 78 (1966).
21. Clausen, T., *Anal. Biochem.*, **22**, 70 (1968).
22. England, M. C., and D. T. Mayer, *Exp. Cell Res.*, **12**, 249 (1957).
23. Cairns, J., *Cold Spring Harbor Symp. Quant. Biol.*, **28**, 43 (1963).
24. Britten, R. J., *Carnegie Inst. Washington, Yearbook*, **65**, 87 (1966).
25. Davidson, E. H., and B. R. Hough, *Proc. Nat. Acad. Sci. USA*, **63**, 342 (1969).