Volume 4 Number 9 September 1977 Nucleic Acids Research

Similarity of satellite DNA properties in the order Rodentia

J. A. Mazrimas and F.T. Hatch

Biomedical and Environmental Research Division, Lawrence Livermore Laboratory, University of California, Livermore, CA 94550, USA

Received 5 July 1977

ABSTRACT

We have characterized satellite DNAs from 9 species of kangaroo rat (Dipodomys) and have shown that the HS-α and HS-β satellites, where present, are nearly identical in all species as to melting transition midpoint (Tm), and density in neutral CsCl, alkaline CsCl, and Cs_2SO_4-Ag^+ gradients. However, the MS satellites exist in two internally similar classes. The satellite DNAs from three other rodents were characterized (densities listed are in neutral CsCl). The pocket gopher, Thomomys bottae, contains Th-α (1.713 g/ml) and Th-β (1.703 g/ml). The guinea pig (Cavia porcellus) contains Ca-α, Ca-β and Ca-γ at densities of 1.706 g/ml, 1.704 g/ml and 1.704 g/ml, respectively. The antelope ground squirrel (Ammospermophilus harrisi) contains Am-α, 1.708 g/ml, Am-β, 1.717 g/ml, and Am-γ, 1.707 g/ml. The physical and chemical properties of the alpha-satellites from the above four rodents representing four different families in two suborders of Rodentia were compared. They show nearly identical Tm, nucleoside composition of single strands, and single strand densities in alkaline CsCl. Similar comparisons on the second or third satellite DNAs from these rodents also indicate a close relationship to each other. Thus the high degree of similarity of satellite sequences found in such a diverse group of rodents suggests a cellular function that is subject to natural selection, and implies that these sequences have been conserved over a considerable span of evolutionary time since the divergence of these rodents about 50 million years ago.

INTRODUCTION

The genome of many rodent species contains a rich source of satellite DNAs which consist of short sequences, 3-15 nucleotides long, that were iterated millions of times during the course of evolution. These repeated sequences are rather homogeneous with a low level of base substitutions or insertions and are arranged in tandem. Frequently they can be isolated as high molecular weight molecules from CsCl or Cs_2SO_4 buoyant density gradients.

In examining the DNA of various rodent species, Walker[1] showed that there were significant differences in the quantity and buoyant density values of satellite DNAs in both neutral and alkaline pHs.

Hennig and Walker[2] concluded from alkaline CsCl gradients and reassociation experiments that the satellite DNA from three species of Apodemus showed differences in density, quantity, and rates of reassociation. Sutton and McCallum[3] reported that heteroduplexes of satellite DNA strands from various Mus species melted 20-25° below the native duplex — evidence of considerable mismatching even within the same genus. Diversity of DNA sequences generally increases further as the evolutionary relationships between taxa become more distant as shown by Rice[4] and Rice and Strauss[5].

In contrast to the above findings, multiple satellite DNAs present in a single animal species may have a common origin. Sequence analysis of pyrimidine tracts in the guinea pig by Southern[6,7] showed that three satellites could have arisen by amplification of a short ancestral sequence in a series of multiplication steps with intervening mutations. Gall and Atherton[8] found by sequence analysis that three satellite DNAs in Drosophilia virilis were related to one another by single base substitutions. In D. melanogaster, Peacock et al.[9] showed that the base sequences of three satellites are related to each other by single base substitutions and variable arrangements of AT and AAT sequences. The chimpanzee satellite A cross-hybridizes with human satellite III, indicating some common sequences between two closely related higher primates (Prosser et al.[10]).

Our findings in kangaroo rats, the genus Dipodomys, and in certain other rodents differ from both of the foregoing situations. Within a single species, Dipodomys ordii, the base sequences of the three major satellites are totally unrelated (Salser, et al.[11]). Among related

Table 1

Taxonomy of the Rodents

ORDER: RODENTIA
SUBORDER: SCIUROMORPHA
FAMILY: Geomyidae
GENUS: Thomomys
FAMILY: Heteromyidae
GENUS: Dipodomys
FAMILY: Sciuridae
GENUS: Ammospermophilus
SUBORDER: HYSTRICOMORPHA
FAMILY: Caviidae
GENUS: Cavia

Dipodomys species there is great similarity in some properties of the satellite DNAs (Mazrimas and Hatch[12]). In this report we shall show that the similarity extends in at least one case to rodents in four different families (Table 1). The findings indicate that a particular hexanucleotide sequence has been conserved broadly through the rodent order for about 50 million years. The implications of this work for the evolution of rodent species and for the selective conservation of satellite DNA will be discussed.

MATERIALS AND METHODS

Animal Acquisition

Kangaroo rats (Dipodomys) were trapped in locations and by methods previously described (Mazrimas and Hatch[12]). The antelope ground squirrel Ammospermophilus harrisi was trapped in the Mohave Mts., Mohave Co., Arizona and the pocket gopher Thomomys bottae was trapped in the Berkeley Marina, Alameda Co., CA by Dr. James Mascarello, Vertebrate Zoology Museum, University of California, Berkeley, CA. Guinea pigs (Cavia porcellus) were purchased from a laboratory animal breeder.

Preparation of DNA

DNA was isolated and purified using CsCl buoyant density gradients as described previously (Hatch and Mazrimas[13]).

Centrifugation and Fractionation

Preparative CsCl buoyant density gradients were made from Harshaw Radiochemical grade salt at an initial density of 1.71 g/ml as previously described (Hatch and Mazrimas[13]). All DNA samples used for Cs_2SO_4-Ag^+ buoyant density centrifugation were extensively purified to

Table 2

Conditions for Optimal Separation in Cs_2SO_4 - Ag^+ Density Gradients

SPECIES	INITIAL DENSITY g/ml	MOLAR RATIO Ag^+/DNA-P	µg $AgNO_3$/µg DNA
D. ordii and D. compactus	1.54	0.33	0.175
D. microps, D. panamintinus D. heermanni, D. merriami D. ingens, D. agilis D. stephensi	1.525	0.29	0.154
D. deserti, D. spectabilis	1.523	0.24	0.125
Cavia porcellus	1.525	0.25	0.133
Thomomys bottae	1.525	0.25	0.133
Ammosphermophilus harrisi & A. leucurus	1.520	0.22	0.117

remove all traces of RNA and protein. The samples were prepared for these gradients as previously described, except for adjustments in the initial salt density and molar ratios of Ag^+ to DNA-phosphorus to achieve maximum resolution of the various components (Table 2).

Actinomycin D-CsCl density gradients were utilized to separate the two satellites in the HD peak from a Cs_2SO_4-Ag^+ gradient on DNA of D. ordii. A typical gradient contained about 250 µg of HD DNA, and 125 µg of Actinomycin D adjusted to an initial CsCl density of 1.59 g/ml and centrifuged for 60 hours at 38,000 rpm in a #50 Ti rotor (Spinco). Fractions were collected; the two appropriate peaks were pooled; and the antibiotic was removed by three extractions with isopropanol mixed with saturated CsCl solution. Under these conditions, HS-alpha separated completely from the MS satellite since MS intercalates Actinomycin D and becomes lighter in density.

For the isolation of single strands, DNA samples were centrifuged in CsCl at a density of 1.76 g/ml and pH of 12.6 (adjusted with 1 M NaOH), and containing 1 mM EDTA and 0.1% sodium sarcosinate. Polyallomer tubes, ½ by 2½ in., were prewashed in alkaline detergent to prevent sticking of strands to tube walls. Samples were centrifuged for 24 hrs. in a Spinco 50.3 rotor at 45,000 rpm. Forty fractions of 0.1 ml were collected, diluted 1 x with water and absorbances were read at 260 nm in a Gilford spectrophotometer. Peaks containing separate strands were pooled and dialyzed several times against 0.01 M Tris buffer pH 7.5 containing 0.1 mM EDTA.

Analytical Density Gradient Centrifugation

Analytical centrifuge separations were conducted with Harshaw optical grade CsCl as previously described (Hatch and Mazrimas[13]). Poly (dAT-dAT) was used as a relative density marker in neutral CsCl (p = 1.679 g/ml) and in alkaline CsCl (p = 1.722 g/ml) centrifugations. All centrifugations were for 24 hrs at 40,000 rpm in a Spinco Model E ultracentrifuge and the cells were scanned at 265 nm with an ultraviolet multiplex scanner.

Thermal Denaturation Profiles

Thermal denaturation was performed in a Model 2400 recording spectrophotometer (Gilford Instrument Co.) using the Model 2524 thermo-programmer with thermoelectric heating in a four-cell holder. Melting profiles were monitored at 260 nm while each cell compartment containing 250 µl of DNA solution in 40 mM KCl, 0.5 mM EDTA, pH 7.5 was

heated at the rate of one degree/min. Calculations and plots were made by a computer program as described previously (Hatch and Mazrimas[13]).

DNA Nucleoside Composition

The DNA samples for analysis were dialyzed against 0.2 M ammonium formate buffer, pH 9.0 containing 1 mM magnesium acetate. Approximately 50-100 µg of DNA in 200 µl of dialysis buffer were incubated at 37°C for 5 hr after addition of three enzymes. DNAase I, code:DPFF (10 µg), venom phosphodiesterase, code:VPH (10 µg) and alkaline phosphatase, code:BAFF (5 µg) were added sequentially after determining concentrations of a stock solution of each enzyme by absorbance and extinction coefficients provided by Worthington Biochemical Corp. After incubation the nucleosides were separated from the enzymes in a Bio-gel P-2 (Bio-Rad) column fashioned from a 5½ inch pasteur pipette plugged with glass wool. The nucleosides were eluted with distilled water as described by Uziel[14]. The eluate was concentrated to 25 to 50 µl under nitrogen in preparation for column chromatography.

For separation of the nucleosides, 10 µl of the concentrated nucleoside preparation was injected into a Model 830 Dupont high-pressure liquid chromatograph fitted with a 30 cm. cation exchange column (Bio-Rad, A-7), which was eluted with 0.2 M ammonium citrate, pH 4.5 at a temperature of 55°C. Separation was effected in 30 minutes at 1500 psi. Quantification of the nucleosides was carried out by means of an attached electronic chart integrator or by measurement of peak height (Brown[15]). The response factor for each nucleoside was determined with pure standards purchased from P-L Biochemicals, Inc.

Satellite Nomenclature

In this report we have retained the published designations for the various satellites isolated from the kangaroo rat (Dipodomys), namely, HS-alpha and HS-beta for the two satellites of the 1.713 g/ml peak and MS for the 1.707 g/ml peak (densities in neutral CsCl). For the guinea pig Cavia porcellus, we designate the alpha satellite (satellite I of Corneo et al., 1970) as Ca-alpha, p = 1.706 g.ml[16]. For Thomomys bottae (pocket gopher) the main satellite is designated as Th-alpha and its density in neutral CsCl is p = 1.713 g/ml. For Ammospermophilus harrisi (antelope ground squirrel) the main satellite is designated as Am-alpha, with a density of 1.708 g/ml in CsCl. If second or third satellite components were found in the above animals, they are given the designations beta and gamma.

RESULTS

Dipodomys Interspecies Comparisons

Isolated satellite DNAs from 9 species of kangaroo rat were submitted to alkaline CsCl buoyant density centrifugation in the analytical ultra-centrifuge. The calculated densities of the single strands are given in Table 3. Within experimental error (± 1 mg/ml), the strand densities for a given satellite are nearly identical among species. Two of the satellites (HS-alpha and HS-beta) are virtually identical in each species in neutral CsCl as shown previously (Mazrimas and Hatch[12]) and also in alkaline CsCl. On the other hand, the MS satellite is similar in all neutral CsCl density gradients. But in alkaline CsCl gradients the species can be divided into two types: two species show a compositional strand bias and seven species show no such bias.

Rodent Interfamily Comparisons

Figure 1 shows a comparison of a) analytical neutral CsCl gradients and b) Cs_2SO_4-Ag^+ preparative gradients of the four rodent species; Dipodomys ordii, Cavia porcellus, Thomomys bottae and Ammospermophilus harrisi. In CsCl the alpha satellites are heavier than main band DNA, indicating possibly a G-C rich DNA species. In the series of preparative Cs_2SO_4-Ag^+ density gradients the alpha satellites of the four species are heavy (bind Ag^+) and appear in similar positions relative to the main band DNA. Careful pooling of gradient fractions of the heavy density satellites from 3 species results in nearly pure satellite fractions, whereas a subsequent Actinomycin D-CsCl gradient is required to separate the MS and HS-alpha satellites from the kangaroo rat.

Table 3

Interspecies Comparison of Kangaroo Rat Satellites
Alkaline CsCl Strand Densities (g/ml)

	Satellite		
SPECIES	HS-α	HS-β	MS
D. ordii[a]	1.709, 1.796	1.762, 1.771	1.737, 1.759
D. compactus	1.709, 1.798	1.761, 1.771	1.738, 1.758
D. microps	1.709, 1.795	1.763, 1.772	1.754
D. panamintinus	1.710, 1.795	1.762, 1.770	1.751
D. herrmanni	1.709, 1.795	1.762, 1.773	1.755
D. merriami	1.708, 1.796	--- ---	1.758
D. ingens	1.709, 1.796	1.762, 1.771	1.752
D. agilis	1.710, 1.796	1.762, 1.771	1.752
D. stephensi	1.712, 1.794	1.762, 1.772	1.762

[a](ref 13).

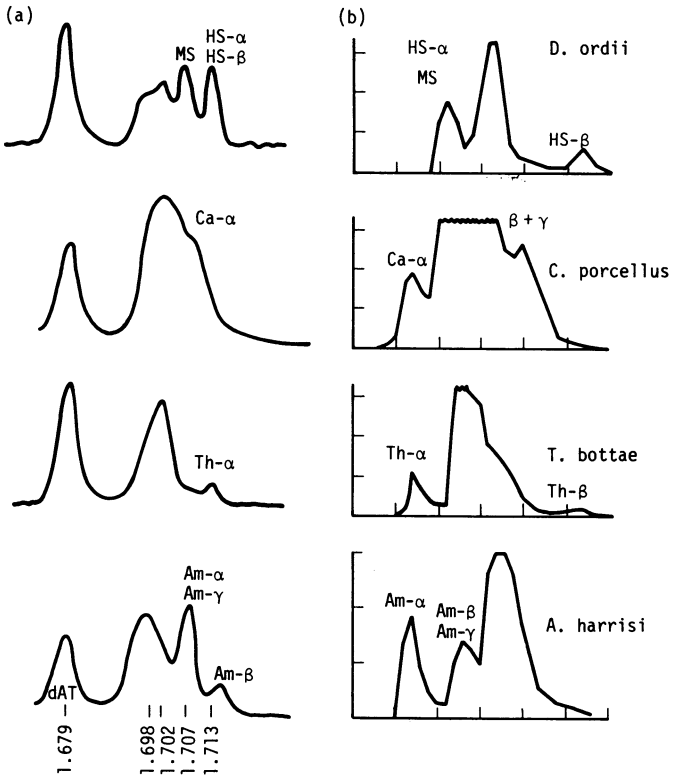

Figure 1. Analytical and Preparative Cs Salt Buoyant Density Gradients of Four Rodent Species.
a. Neutral CsCl analytical gradient;
b. Cs_2SO_4-Ag^+ preparative gradient. Tick marks indicate five-fraction intervals.

Physical properties of the alpha satellites are summarized in Table 4. Analytical density gradients in alkaline CsCl on the four purified heavy density alpha satellites showed nearly identical strand bias in the kangaroo rat and pocket gopher and somewhat different strand bias in the guinea pig and antelope ground squirrel. These values presumably reflect the similar strand asymmetries in nucleoside composition (Table 5). The density values of the single strands of Ca-α reported here differ somewhat from values published by other investigators[16]. The reasons for these discrepancies are unknown; however, our values are reproducible with different isolations of this satellite DNA. The heavy strands are consistently low in deoxycytidine

Table 4

Summary of Physical Properties of the Alpha Satellites

SPECIES	neutral CsCl g/ml	alkaline CsCl g/ml	Tm °C	G + C mole percent
Dipodomys ordii	1.713	1.709, 1.796	77.5°	40.3
Thomomys bottae	1.713	1.708, 1.795	76.2°	37.9
Cavia porcellus	1.706	1.704, 1.786	75.5°	36.4
Ammospermophilus harrisi	1.708	1.701, 1.793	75.8°	36.7

Table 5

Base Composition of Single Strands of Alpha Satellites
(mole percent)

	H strand	L strand	Duplex
HS-alpha (Dipodomys ordii)[a]			
dT	38.4	23.1	30.8
dG	34.8	5.0	19.9
dA	23.4	33.3	28.4
dC	3.0	37.7	20.4
Th-alpha (Thomomys bottae)			
dT	40.3	26.4	33.4
dG	31.9	4.4	18.2
dA	22.2	36.0	29.1
dC	5.8	33.6	19.7
Ca-alpha (Cavia porcellus)			
dT	42.8	23.4	33.1
dG	30.9	6.8	18.9
dA	23.0	39.0	31.0
dC	4.0	31.0	17.5
Am-alpha (Ammospermophilus harrisi)			
dT	42.5	23.2	32.8
dG	32.6	1.9	17.3
dA	23.4	37.3	30.4
dC	2.0	36.9	19.4

[a]See also ref. 13

and high in deoxythymidine. Conversely, the light strands are low in deoxyguanosine and high in deoxyadenosine. The content of 5-methyldeoxycytidine is not reported because it was less than 0.5 mole percent, a level at which it is difficult to obtain accurate data on the limited amounts of these fractions that were available. The four alpha satellites were dialyzed against 40 mM KCl-0.5 mM EDTA and thermally denatured (Figure 2). Their transition midpoints are proportional to the

G + C content of the satellites.

The foregoing results show a close similarity among alpha satellites from four rodents from different families. It was therefore of interest to look for similarities among any additional satellites present. Table 6 gives an interspecies comparison of certain properties of these additional satellites. Firstly, there appears to be close

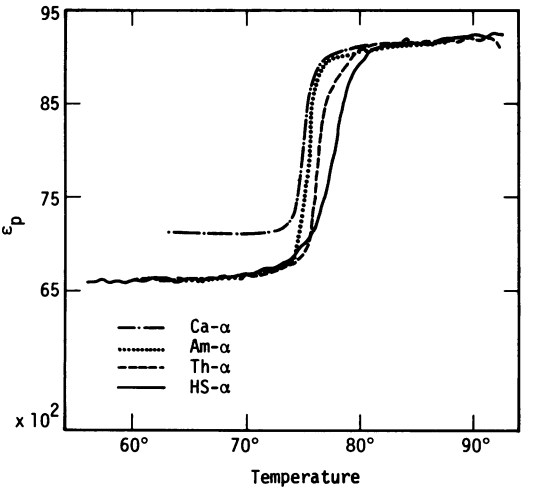

Figure 2. Thermal Ultraviolet Denaturation Curves of Alpha Satellite DNAs from Four Rodent Species.
The solvent was 40 mM KCl, 0.5 mM EDTA, pH 7.5

Table 6

Interspecies Comparison of Other Satellite DNAs

Species	Satellite	Percent of Genome	Buoyant Density Neutral CsCl g/ml	Buoyant Density Alkaline CsCl g/ml	Tm °C	Base Sequences
Kangaroo Rat	HS-β	11	1.713[a]	1.762, 1.771[a]	85.7°	ACACAGCGGG[b]
Antelope Ground Squirrel	Am-β	9	1.717	1.763, 1.777	82.5°	Unknown
Kangaroo Rat	MS	22	1.707[a]	1.737, 1.759[a]	77.7°	AAG, CAG[b]
Guinea Pig	Ca-β	2.5	1.704	1.738, 1.769	78.5°	AAG, AGA, GAA[c]
Guinea Pig	Ca-γ	2.5	1.704	1.741, 1.762	78.5°	
Pocket Gopher	Th-β	3	1.702	1.730, 1.769	77.3°	Unknown
Antelope Ground Squirrel	Am-γ	13	1.707	1.728, 1.763	77.5°	Unknown

[a] Ref. 13
[b] Ref. 11
[c] Ref. 7

homology between HS-beta and Am-beta in buoyant densities in neutral and alkaline CsCl and in Tm. These similarities may indicate that these satellites contain related base sequences. Secondly, MS, and Ca-beta and gamma, and likewise Th-beta and Am-gamma seem to be closely related in physical properties.

DISCUSSION

Members of the rodent order are the most successful mammals in occupying a large portion of the world's land areas in almost every type of ecological niche known to man. Today there are 35 extant families that comprise the order Rodentia, although fossil evidence indicates that several additional families became extinct. About 25 families are known from the Eocene (approximately 50 million years ago) and it appears that this basic order arose from allopatric speciation leading to many independent basal lineages (Simpson[17]). During the course of evolution, many similar and specialized adaptations appeared independently in the numerous lineages indicating the widespread operation of both parallel and convergent mechanisms of evolution throughout the entire diverse order of rodents.

We wish to address a series of questions concerning the properties of satellite DNA in rodents. (1) Are any "rules" emerging concerning these properties that provide clues to their biological role? From research in a number of laboratories on a variety of rodents the predominant feature of satellite DNA is variation. This variation includes the chemical properties to be discussed in response to the following questions as well as the timing of S-phase replication (Bostock, et al.[18]) and the chromosomal localization (Prescott, et al.[19]; Bostock and Christie[20,21]). Thus far each new study has revealed exceptions to the apparent "rules" generated in previous work.

(2) Do similar satellites present in several species within a genus arise independently or from a common original sequence? Earlier findings in Apodemus species by Hennig and Walker[2] showed sufficient variation in buoyant densities and reassociation properties that the satellite origins were considered to be independent. However, in the present study of Dipodomys species we found a high degree of interspecies similarity in neutral and alkaline CsCl buoyant densities for satellite DNAs.

(3) What is the significance of the marked interspecies similarity of analogous satellite DNAs among phylogenetically diverse

rodents? This finding is the principal subject of the present article and of one by Fry and Salser[22]. The evidence presented in this paper consists of similarities in base composition, Tm, and the buoyant densities of single DNA strands in alkaline CsCl. We believe that the single strand densities are the most significant data, by virtue of their marked dependence on base composition[23]. It would appear that alteration of any single base in a highly reiterated simple sequence of ten or less nucleotides would have a substantial effect on strand densities. However, there appears to be no published data on the effect of sequence isomers (having identical base composition) on strand density, so we cannot rule out isomeric differences between the satellites on the basis of these data. Stronger support for the near identity of the alpha satellites from different rodents comes from the fingerprint and partial sequence data of Fry and Salser[22]. Not only do the major repeating sequences of these satellites appear to be the same, but there is also a high degree of similarity of the most common sequence variants.

Whereas it is reasonably easy to accept the occurrence of parallel or convergent evolution of anatomical characters and environment-related coat color among diverse rodents, the convergent or coincidental reiteration of nearly identical simple noncoding base sequences does not seem so likely. Therefore, we retreat to the presumption that particular simple sequences, which were somehow readily reiterated in a saltatory manner (Southern[24]) or were subject to unequal crossing over (Smith[25]), existed in ancestral species common to a variety of modern rodent species. The concept of a "library" of satellite precursor sequences has been proposed by Salser *et al.*[11]. If any of these mechanisms was responsible for generating the alpha satellites, then

Table 7

Evolution of the Four Families of Rodents

Epoch	Family	Time Family Established (million years ago)	Satellite DNA (percent of total)
Pliocene	*Caviidae*	2-7	10
Miocene	*Geomyidae*	7-26	13
Oligocene		26-38	
Eocene	*Heteromyidae*, *Sciuridae*	38-54	52,43

conservation by natural selection seems an essential feature to account for the marked similarity observed in the several modern species. This would, in turn, imply that the sequences possess a function affecting the longevity or fecundity of the species since their common origin in the Eocene about 50 million years ago (Table 7). A possible role of satellite DNAs in promoting karyotype rearrangement and the evolution of species has recently been discussed by the authors[26].

ACKNOWLEDGEMENTS

This work was sponsored by the U.S. Energy Research and Development Administration (Contract No. W-7405-ENG-48). Reference to a company or product name does not imply approval or recommendation by the U.S.E.R.D.A. or the University of California to the exclusion of others that may be suitable.

We thank Dr. James Mascarello for helpful discussions and for providing specimens of Ammospermophilus and Thomomys.

REFERENCES

1. Walker, P.M.B. (1968). Nature 219, 228-232.
2. Hennig, W., and Walker, P.M.B. (1970). Nature 225, 915-919.
3. Sutton, W.D., and McCallum, M. (1972). J. Mol. Biol. 71, 633-656.
4. Rice, N.R. (1972). Evolution of Genetic Systems, Brookhaven Symp. in Biology No. 23, ed. Smith, H.H. pp. 44-79, Gordon and Breach, New York.
5. Rice, N.R. and Straus, N.A. (1970) Proc. Nat. Acad. Sci. (U.S.) 3546-3550.
6. Southern, E.M. (1970) Nature 277, 794-798.
7. Southern, E.M. (1973) Modern Aspects of Cytogenetics: Constitutive Heterochromatin in Man. ed. R.A. Pfeiffer pp. 19-27, Schattauer Verlag, New York.
8. Gall, J.G. and Atherton, D.D. (1974) J. Mol. Biol. 85, 633-664.
9. Peacock, W.J., Brutlag, D., Goldring, E., Appels, R., Hinton, C.W. and Lindsley, D.L. (1973) Cold Spring Harbor Symp. Quant. Biol. 38, 405-416.
10. Prosser, J., Moar, M., Bobrow, M. and Jones, K.W. (1973) Biochim. Biophys. Acta 319, 122-134.
11. Salser, W., Bowen, S., Browne, D., El Adli, F., Fedoroff, N., Fry, K., Heindell, H., Paddock, G., Poon, R., Wallace, B. and Whitcome, P. (1976) Federation Proc. 35, 23-35.
12. Mazrimas, J.A. and Hatch, F.T. (1972) Nature New Biol. 240, 102-105.
13. Hatch, F.T. and Mazrimas, J.A. (1974) Nucleic Acid Res. 1, 559-575.
14. Uziel, M. (1967) Meth. Enzymol. 12A, 407-414.
15. Brown, P.R., Bobick, S. and Hanley, F.L. (1974) J. Chromatog. 99, 587-595.
16. Fasman, G.D. (1976) CRC Handbook of Biochemistry and Molecular Biology, 3rd edn. Vol II, p 394, CRC Press, Ohio.

17. Simpson, G.G. (1959) Cold Spring Harbor Symp. Quant. Biol. 24, 255-271.
18. Bostock, C.J., Christie, S., Lauder, I.J., Hatch, F.T. and Mazrimas, J.A. (1976) J. Mol. Biol. 108, 417-433.
19. Prescott, D.M., Bostock, C.J., Hatch, F.T. and Mazrimas, J.A. (1973) Chromosoma (Berl.) 42, 205-213.
20. Bostock, C.J. and Christie, S. (1974) Chromosoma (Berl.) 48, 73-87.
21. Bostock, C.J. and Christie, S. (1975) Chromosoma (Berl.) 51, 25-34.
22. Fry, K., Salser, W., Cell, in press.
23. Fasman, G.D. (1976) CRC Handbook of Biochemistry and Molecular Biology, 3rd edn. Vol I p. 572 CRC Press, Ohio.
24. Southern, E.M. (1974) Eukaryotic DNA, International Review of Science, Vol. 6, Biochemistry of Nucleic Acids, ed. K. Burton, pp. 101-139, University Park Press, Baltimore.
25. Smith, G.P. (1976) Science 191, 528-535.
26. Hatch, F.T., Bodner, A.J., Mazrimas, J.A. and Moore, D.H.,II (1976) Chromosoma (Berl.) 58, 155-168.