THE BASE COMPOSITION OF RIBONUCLEIC ACID IN LAMPBRUSH CHROMOSOMES, NUCLEOLI, NUCLEAR SAP, AND CYTOPLASM OF *TRITURUS* OOCYTES

J. -E. EDSTRÖM, M.K., and J. G. GALL, Ph.D.

From the Department of Histology, University of Gothenburg, Sweden, and the Department of Zoology, University of Minnesota, Minneapolis

ABSTRACT

The base composition of RNA's extracted from chromosomes, nucleoli, nuclear sap, and cytoplasm of *Triturus* oocytes has been determined by microelectrophoresis. The chromosomal RNA has a content of guanine+cytosine equal to that of DNA, but there is no complementarity in the composition as for DNA. Nuclear sap contains a highly variable RNA with a tendency towards high uracil values. Nucleolar and cytoplasmic RNA's are similar in composition and both are of the guanine-cytosine rich type. The chromosomes and nucleoli contain roughly equivalent amounts of RNA, somewhat less than is present in the nuclear sap. The RNA/DNA ratio of the whole chromosomes is about 10. However, the ratio in the synthetically active regions, the loops, is much higher, since the loops contain all the chromosomal RNA but only a small fraction of the DNA.

RNA[1] synthesis in isolated thymus nuclei was found to be DNA dependent (Allfrey and Mirsky, 1962) and a similar dependence may apply to all cellular RNA synthesis (Hurwitz *et al.*, 1962; also for references). The restriction of DNA to the chromosomes gives chRNA a unique position since it should contain RNA newly synthesized under the influence of DNA. Chromosomes with acceptable identity, integrity, and purity are not easily available for conventional biochemical techniques. It is possible, however, with suitable material and microchemical methods to investigate RNA from cell parts isolated by micromanipulation under microscopic control. The dipteran giant chromosomes represent one such favorable case (Edström and Beermann, 1962), lampbrush chromosomes from amphibian oocytes another one. Analyses of the RNA from lampbrush chromosomes and other constituents of the oocyte will be reported.

[1] Abbreviations: RNA = ribonucleic acid, DNA = deoxyribonucleic acid, chRNA = chromosomal RNA, A = adenine, G = guanine, C = cytosine, U = uracil.

METHODS

Biological Material

Nuclei were isolated manually from oocytes of *Triturus viridescens* and *Triturus cristatus carnifex* in a medium consisting of 5 parts 0.1 M KCl and one part 0.1 M NaCl. In some cases a trace of calcium (0.5×10^{-4} M $CaCl_2$) was added. The nuclear envelope was removed with forceps and the nuclear contents allowed to spread on a coverslip (Fig. 1). The material was fixed for 15 minutes to several hours in the vapor of unbuffered formalin or of formalin containing 1 per cent acetic acid (Gall, 1963 *a*). In most cases, the nucleoli and chromosomes attach to the

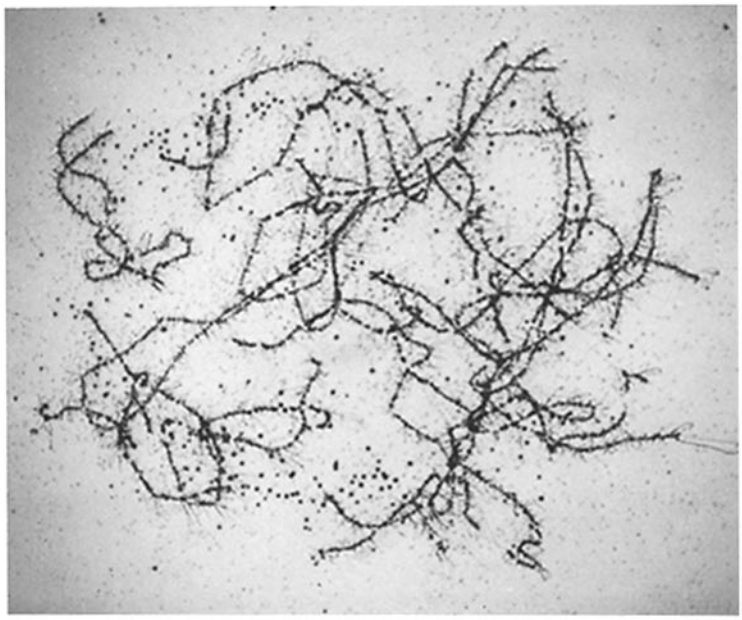

FIGURE 1 Chromosomes and nucleoli isolated from an oocyte nucleus of the newt, *Triturus*. The nucleoli are the small granules especially numerous at bottom center and upper left. Fixed and dried preparation similar to those used for analyses. × 65.

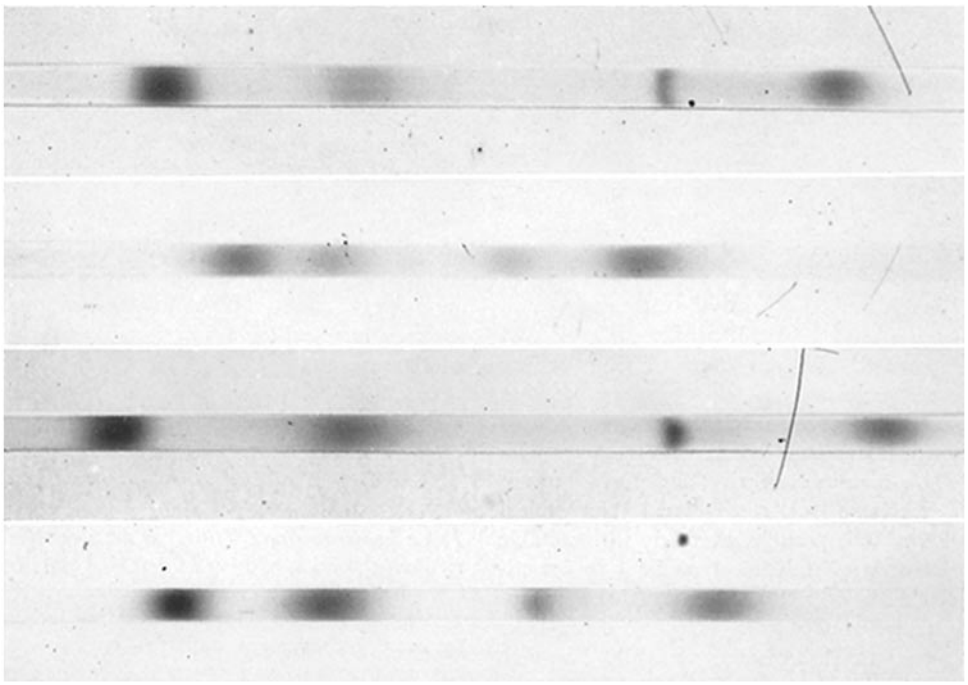

FIGURE 2 Microphoretic separations of RNA extracted from *Triturus* oocytes, photographed at 257 mμ. From top to bottom: chromosomes, nuclear sap, nucleoli, and cytoplasm. From left to right the separated zones represent adenine, guanine, cytidylic acid, and uridylic acid, respectively. × 170.

coverslip before the nuclear sap precipitates. By rinsing at the appropriate time one obtains preparations essentially free of sap. After longer fixation the nuclear sap precipitates as a mass on or near the chromosome group. The chromosomes and nucleoli can be scraped away to provide samples of pure sap material. Bits of oocyte cytoplasm were removed with a pipette and fixed on coverslips in the vapor of unbuffered formalin. After fixation the preparations were rinsed in water, dehydrated in an ethanol series, passed through xylene and acetone, and finally air dried. Coverslips bearing the dried material were prepared by one of us (J. G. G.) and sent by air to the other (J. -E. E.) for analysis.

Chemical analyses

Cover glasses with isolated material were inverted in an oil chamber arrangement and handled with microinstruments maneuvered by a de Fonbrune micromanipulator. The material was wetted with 0.01 N acetic acid and afterwards arranged according to needs with two glass needles. Nucleoli were collected individually and placed in groups; chromosomes were scraped off the glass surface and those belonging to the same nucleus collected as one sample. Material from one set was used for 1–3 analyses; in a few cases material pooled from two cells was used. Nuclear sap, precipitated on the glass surface, was treated in the same way. Finally, pieces of precipitated cytoplasm were used, from which RNA in amounts for several analyses could be extracted. RNA extractions, determination of RNA content in the extracts, hydrolysis, and microelectrophoretic separations (microphoresis) were carried out as previously described (summarized in Edström, 1963). Typical microphoretic separations are shown in Fig. 2.

Determinations of the A/G ratio in DNA as well as the DNA content per diploid nucleus were performed by microphoresis. For these the purines of isolated erythrocyte nuclei were extracted in the oil chamber with 1 N HCl for 18 hours. Results on *T. c. carnifex* and *T. viridescens* will be given, but complete data together with values for other amphibia will be published separately.

RESULTS

Relation between the Quantities of RNA in Nucleoli, Nuclear Sap, and Chromosomes

It is known that the size of the loops in the lampbrush chromosomes as well as the number and size of the nucleoli vary during the growth of the oocyte (Callan and Lloyd, 1960). Data on the total RNA content of the nuclear constituents apply, therefore, only to the stages analyzed. In *T. c. carnifex* oocytes of about 1 mm diameter, 2000 μμg RNA is localized in the nucleoli and three times as much in the combined nuclear sap and chromosomes. RNA for one or two analyses, *i.e.*, about 1000 μμg, can be recovered from the chromosomes, but since it is difficult to collect the chromosomes quantitatively, the amount of chRNA probably lies in the range of 1000 to 2000 μμg (Table I). The diploid or 2C amount of DNA is 67 μμg for this species (*vide infra*). Assuming that the oocyte chromosomes contain the 4C amount, we may estimate the RNA/DNA ratio to be about 10. However, the ratio is very much higher, probably closer to 200, in the active parts of the chromosomes—the loops—since the loops represent only a small fraction of the total chromatid length (Gall and Callan, 1962).

TABLE I

RNA Content in Nuclear Constituents of Oocytes (diameter about 1 mm) from Triturus cristatus carnifex

	RNA content	μμg RNA per nucleolus	n
	(μμg)		
Chromosomes	1490, 640	—	2
Nucleoli	1700–2750	4.6–10.0	4
Chromosomes + sap	5160–8250	—	6
Sap (by difference)	4000–6000	—	—

DNA Content and Composition in Triturus Erythrocytes

The DNA content per erythrocyte nucleus is 67 μμg for *T. c. carnifex* and 89 μμg for *T. viridescens*. The A/G ratios are 1.25 and 1.23, respectively (difference insignificant), corresponding to guanine + cytosine (G-C) contents of 44.4 per cent and 44.8 per cent.

Chromosomal RNA

The general feature of chRNA in both species is a relatively low G-C content, 45.8 per cent for *T. c. carnifex* and 44.1 per cent for *T. viridescens*, close to that of the DNA. However, chRNA has individual base ratios deviating from those of DNA, *viz.*, too little adenine and guanine and too

much uracil and cytosine (Table II). The deviations from complementarity with regard to the A-U and G-C pairs are far too high to be caused by any errors in the method.

Nuclear Sap RNA

In contrast to the other constituents usually only a minor part of the nuclear sap was recovered for the variable oocytes, it is possible that the composition in the sap is dependent on oocyte size. A situation like the present one could arise if the sap consists of two fractions: a uniform G-C rich fraction, increasing in amount with oocyte size (nucleolar origin), and a variable, U-rich fraction, unrelated in amount to the size of the cell (functional messenger).

TABLE II

Base Composition of RNA from Individual Chromosome Sets from Triturus

Mean values of molar proportions in per cent of the sum ±S.E.M. n = number of samples = number of chromosome sets, except for one analysis of *T. viridescens* where two sets were pooled.

	Adenine	Guanine	Cytosine	Uracil	n
Triturus cristatus carnifex	26.0 ± 0.45	20.6 ± 0.24	25.2 ± 0.55	28.3 ± 0.29	10
Triturus viridescens	26.6 ± 0.52	20.4 ± 0.52	23.8 ± 0.67	29.3 ± 0.54	10

TABLE III

Base Composition of RNA Extracted from Samples of Nuclear Sap from Triturus cristatus carnifex

Mean values of molar proportions in per cent of the sum ± S.E.M.

Oocyte No.	Adenine	Guanine	Cytosine	Uracil	Oocyte diameter
					(μ)
19	23.7	23.2	26.6	26.5	780
20	25.3	22.7	25.5	26.6	840
21	25.0	23.1	27.6	24.4	1260
43 + 44	22.2	18.4	28.2	31.2	600, 650
45	18.3	16.2	22.4	43.1	700
47 + 48	18.1	11.3	21.1	49.5	700, 700
51	27.2	18.9	21.0	32.9	650
52	24.5	18.8	23.4	33.4	700
53	23.1	21.1	23.7	32.1	700
54	22.0	17.7	25.0	35.3	750
55	23.8	20.1	26.3	29.9	1500
56	23.0	20.2	24.9	32.0	1500
57	25.1	23.0	23.9	27.9	1500

base analysis. Values on the sap, therefore, may not be representative of the average base composition of all the sap RNA. Three samples of sap from *T. c. carnifex* in one lot (oocytes Nos. 19 to 21) exhibited relatively uniform and equimolecular values (Table III). In a second lot (Nos. 43 to 57) there was more variation and the U values were high as a rule. In this group, however, sap from the big oocytes, Nos. 55 to 57, contained RNA which was rather similar to that of the first group. Since the oocytes Nos. 19 to 21 were bigger than

Nucleolar and Cytoplasmic RNA

It is known from other material that nucleolar and cytoplasmic RNA's have a similar gross base composition. For *Triturus* there is also an over-all resemblance between nucleolar and cytoplasmic RNA's, and both are of the G-C rich type (Table IV). Although there are small deviations, the two kinds of RNA do not differ in a regular manner, in contrast to the conditions in the starfish oocyte (Edström *et al.*, 1961).

TABLE IV

Base Composition of RNA from Nucleoli and Cytoplasm from Triturus Oocytes

Mean values of molar proportions in per cent of the sum. n_1 = number of samples, n_2 = number of analyses.

Species	Cell component	Oocyte No.	Adenine	Guanine	Cytosine	Uracil	n_1	n_2
Triturus cristatus carnifex	Nucleoli	9–16	18.1	31.7	28.7	21.7	8	23
	Cytoplasm	27, 28	20.1	27.2	29.5	23.0	2	19
Triturus viridescens	Nucleoli	35–42	21.5	29.3	30.1	19.1	7	10
	Cytoplasm	33, 34	23.7	28.0	27.7	20.7	2	10
	Nucleoli	82–95	21.8	27.6	27.8	22.7	7	7
	Cytoplasm	96	20.9	30.2	27.5	21.4	1	3

TABLE V

RNA Content in Individual Purkinje Nerve Cell Bodies of the Cerebellar Cortex after Fixation in Carnoy's Liquid and Neutral Buffered Formalin

Mean values ± S.E.M.

	Carnoy fixation	Formalin fixation
$\mu\mu$g RNA	146 ± 9 (n = 5)	131 ± 6 (n = 5)

TABLE VI

Base Composition of RNA Extracted from Freeze-Dried Starfish Ovaries after Different Treatments

Molar proportions in per cent of the sum.

	Direct determination	Carnoy fixation	Formalin fixation
Adenine	18.6	19.9	20.6
Guanine	33.4	31.9	31.2
Cytosine	27.9	25.5	27.6
Uracil	20.1	22.7	20.6

Control Experiments

Adjoining pieces of rat cerebellum were fixed in Lillie's buffered formalin for 3 hours or in Carnoy's fixative (ethanol, chloroform, acetic acid, 6:3:1, by volume) for 90 minutes. The latter fixative is known to precipitate the nucleic acids quantitatively and allow complete enzymic extraction of RNA (Edström *et al.*, 1961). The RNA content determined for individual Purkinje nerve cell bodies was practically the same in the two groups (Table V). Formalin fixation was also without effect on the RNA base composition. Freeze-dried pieces of starfish ovaries, fixed in Lillie's buffered formalin, in Carnoy, or untreated were analyzed for RNA base composition. The data of the three groups agreed reasonably well (Table VI). Complete removal of RNA was indicated by the absence of basophilia in all the extracted material when stained with methylene blue at pH 4.6.

During the present work the fixation was shifted from unbuffered to acid formalin (see under *Methods*). No systematic effect was found on RNA.

DISCUSSION

Incorporation experiments which demonstrate an early labeling of the RNA in the chromosomes provide the main experimental arguments for a local origin of chRNA (McMaster-Kaye and Taylor, 1958; Pelling, 1959; Rho and Bonner, 1961). The findings of Gall and Callan (1962) on uridine incorporation and RNA movement in one of the giant loops of the lampbrush chromosomes would be difficult to reconcile with a non-chromosomal origin of chRNA. With consideration of the restriction of DNA to the chromosomes, these results are in accord with the previously quoted findings that nuclear RNA synthesis is DNA dependent. The present results, showing that the G-C content is similar in the two nucleic acids, agree with this view. It is not excluded, however, that the original synthetic product becomes modified. It may be that chRNA is equivalent neither to "informational RNA" in the sense of Hayashi and Spiegelman (1961), *i.e.*, newly synthesized, non-ribosomal, and DNA-like RNA, nor to the functional messenger which participates in protein

synthesis. It cannot constitute a complete copy of both DNA strands, but the G-C content is that expected for a copy of a single DNA strand of average G-C content. The functional messenger seems to be produced by a single DNA strand (Champe and Benzer, 1962), but it is dubious whether it can be identified with chRNA, since the messenger is expected to display a high U/A ratio (Lanni, 1962). RNA fractions with a DNA-like composition and with a high turnover have been isolated previously from animal nuclei (Sibatani *et al.*, 1962; Georgiev and Mantieva, 1962). Their relation to the present chRNA is unknown.

In *Chironomus tentans* salivary gland cells, chRNA is asymmetric with a high A content and a G-C value considerably above that of DNA (Edström and Beermann, 1962). The similarity of the G-C value in chRNA and DNA of *Triturus*, therefore, may be fortuitous. On the other hand, the chRNA of *Triturus* is probably a better average of the gene products than the chRNA of *Chironomus*. In *Triturus* many hundreds of genes appear to be active, whereas only relatively few are active in *Chironomus*.

The significance of the wide variations in base composition of the nuclear sap RNA is not understood. Great variations were also found for the combined chromosome + nuclear sap fraction of Carnoy-fixed starfish oocytes (Edström, unpublished).

A high degree of correlation in base ratios (though not necessarily a complete agreement) between nucleolar and cytoplasmic bulk RNA's has been observed previously for different kinds of material (Edström, 1960; Edström *et al.*, 1961; Edström and Beermann, 1962), supporting the contention that nucleolar RNA is a precursor of ribosomal RNA. This correlation is again observed for *Triturus* with deviations of a few per cent in some cases. The base data agree with the view that the multiple free nucleoli of amphibian oocytes are homologous to the more typical nucleoli associated with the chromosomes in somatic cells of the same species (Gall, 1954, 1963 *b*).

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REFERENCES

ALLFREY, V. G., and MIRSKY, A. E., 1962, *Proc. Nat. Acad. Sc.*, **48**, 1590.

CALLAN, H. G., and LLOYD, L., 1960, *Phil. Tr. Roy. Soc., London, Series B*, **243**, 135.

CHAMPE, S. P., and BENZER, S., 1962, *Proc. Nat. Acad. Sc.*, **48**, 532.

EDSTRÖM, J.-E., 1960, *J. Biophysic. and Biochem. Cytol.*, **8**, 47.

EDSTRÖM, J.-E., 1963, *in* Methods in Cell Physiology, (D. Prescott, editor), New York, Academic Press, Inc., in press.

EDSTRÖM, J.-E., and BEERMANN, W., 1962, *J. Cell. Biol.*, **14**, 371.

EDSTRÖM, J.-E., GRAMPP, W., and SCHOR, N., 1961, *J. Biophysic. and Biochem. Cytol.*, **11**, 549.

GALL, J. G., 1954, *J. Morphol.*, **94**, 283.

GALL, J. G., 1963 *a*, *in* Methods in Cell Physiology, (D. Prescott, editor), New York, Academic Press, Inc., in press.

GALL, J. G., 1963 *b*, *in Growth Symposia*, **21**, 119.

GALL, J. G., and CALLAN, H. G., 1962, *Proc. Nat. Acad. Sc.*, **48**, 562.

GEORGIEV, G. P., and MANTIEVA, V. L., 1962, *Biochim. et Biophysica Acta*, **61**, 153.

HAYASHI, M., and SPIEGELMAN, S., 1961, *Proc. Nat. Acad. Sc.*, **47**, 1564.

HURWITZ, J., FURTH, J. J., MALAMY, M., and ALEXANDER, M., 1962, *Proc. Nat. Acad. Sc.*, **48**, 1222.

LANNI, F., 1962, *Proc. Nat. Acad. Sc.*, **48**, 1623.

MCMASTER-KAYE, R., and TAYLOR, J. H., 1958, *J. Biophysic. and Biochem. Cytol.*, **4**, 5.

PELLING, C., 1959, *Nature*, **184**, 655.

RHO, J. H., and BONNER, J., 1961, *Proc. Nat. Acad. Sc.*, **47**, 1611.

SIBATANI, A., DE KLOET, S. R., ALLFREY, V. G., and MIRSKY, A. E., 1962, *Proc. Nat. Acad. Sc.*, **48**, 471.