

Table 2. Autoradiographic studies of 13-15/21 translocations in Down's syndrome.

Reference	No. of cases with translocation involving chromosome No.		
	13	14	15
This study	0	18	2
Schmid (5)	0	2	0
Yunis <i>et al.</i> (7)	0	2	0
Mikkelsen (7)	0	5	1
Bloom and Gerald (7)	0	3	0
Total	0	30	3

results of previous autoradiographic studies of 13-15/21 translocations in Down's syndrome are similar: no case with chromosome 13, 12 cases with chromosome 14, and one case with chromosome 15 in the translocation (5, 7). Combining these data, we find that there were no translocations with chromosome 13, 30 with chromosome 14, and three with chromosome 15 (Table 2). If the expectation had been that the participation of chromosomes 13, 14, and 15 is random, one would have expected chromosome 13 to have been found in 11 cases, chromosome 14 in 11 cases, and chromosome 15 in 11 cases. The differences between these expectations and the observed data are statistically very highly significant (8), indicating that the entry of chromosomes 13-15 into centric-fusion translocation with chromosome 21 is nonrandom (9).

The factors underlying this nonrandomness are not yet defined. It cannot be related simply to differences in size or shape of chromosomes 13-15, since, as mentioned, these chromosomes are all acrocentrics of similar size. The nonrandomness might reflect different tendencies for broken chromosomes 13, 14, and 15 to fuse with chromosome 21, perhaps because of spatial relationships within the nucleus. The nonrandomness may also reflect differences in the frequencies with which chromosomes 13, 14, and 15 break near the centromere, due perhaps to differences in molecular organization, as suggested by the late replication of the area around the centromere in chromosome 14 and the early replication of that area in chromosomes 13 and 15 (5, 6).

Twelve of the translocations in Table 2 were known to be familial. In no case was a 13/21 translocation demonstrated. As Mikkelsen has noted (see 7), 13-trisomies have not been observed "... in the quite numerous families with 13-15/21 translocations

that have been published. 13/21 translocations may not occur at all and therefore not give rise to the occurrence of 13 trisomy cases. . . ."

It could be argued that chromosome 13 enters into centric-fusion translocations with chromosome 21, but the resultant translocations are not observed in individuals with Down's syndrome. However, in trisomy 13 syndrome a deficiency of 13/21-22 translocations compared to 13/13-15 translocations has been found (3), providing further evidence that 13/21 translocations tend not to form.

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#### References and Notes

1. W. M. Court-Brown, *Human Population Cytogenetics* (North-Holland, Amsterdam, 1967), p. 94.
2. Although there is debate as to whether the small acrocentric chromosome triplicated entirely or partially in Down's syndrome (mongolism) is No. 21 or 22, it will be termed No. 21 here, in keeping with conventional practice.

3. F. Hecht, R. E. Magenis, R. B. Lyons, H. Thompson, *Ann. Génét.* **9**, 155 (1966).
4. This is inferred by reports of phenotypically normal individuals with deletion of: the short arm of a 13-15 chromosome [J. de Grouchy, C. Salmon, D. Salmon, P. Maroteaux, *Ann. Génét.* **9**, 80 (1966)], the short arm of chromosome 13 [W. Bias and B. R. Migeon, *Am. J. Human Genet.* **10**, 393 (1967)]; and the short arms of chromosomes 13 and 14 due to 13/14 centric-fusion translocation [J. J. Yunis, M. Alter, E. B. Hook, M. Mayer, *New Engl. J. Med.* **271**, 1133 (1964); F. Giannelli and R. M. Howlett, *Cytogenetics* **5**, 186 (1966)].
5. W. Schmid, *Cytogenetics* **2**, 175 (1963).
6. J. J. Yunis, E. B. Hook, M. Mayer, *Lancet* **2**, 935 (1964); J. L. German, in *Cytogenetics of Cells in Culture*, R. J. C. Harris, Ed. (Academic Press, New York, 1964), p. 191; F. Giannelli, *Nature* **208**, 669 (1965); W. Gey, *Humangenetik* **2**, 246 (1966). Yunis and co-workers refer to chromosomes 13, 14, and 15 as D<sub>1</sub>, D<sub>2</sub>, and D<sub>3</sub>.
7. J. J. Yunis, E. B. Hook, M. Mayer, *Am. J. Human Genet.* **17**, 191 (1965); M. Mikkelsen, *Ann. Human Genet.* **30**, 325 (1967); G. E. Bloom and P. S. Gerald (abstract), Meeting of American Society for Human Genetics, 1-3 Dec. 1967, Toronto.
8. The chances are as follows for finding no case with chromosome 13 (chi-square 16.5,  $P < .001$ , 1 d.f.), 30 cases with chromosome 14 (chi-square 49.2,  $P < .001$ , 1 d.f.), and 3 cases with chromosome 15 (chi-square 8.7,  $P < .005$ , 1 d.f.).
9. The entry of chromosomes 13-15 into centric-fusion translocation with other chromosomes in the 13-15 group may also prove to be nonrandom. Autoradiographic analysis by several laboratories of five cases show all to be 13/14 translocations (G. E. Bloom and P. S. Gerald, Meeting of Society for Pediatric Research, 3-4 May 1968, Atlantic City, N.J.).
10. We thank Mrs. M. Webb for cells from patient No. 230/67. This study was supported by NIH grants CA 07941 and 1 TO1 HD00165 and by grants from the Children's Bureau to the Crippled Children's Division, Portland, for a genetics program and to the Children's Hospital, Los Angeles (project No. 422). J.V.H. was on leave from the department of zoology, Michigan State University, and Lapeer State Home and Training Center.

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## Rhaphidosomes: 2'-O-Methylated Ribonucleoproteins

Abstract. *Methylation of the RNA component from seven flexibacterial rhaphidosomes varied in extent, distribution along the RNA chain, and distribution between nucleotides. Flexibacterial soluble and ribosomal RNA had normal (low) methylation.*

Rhaphidosomes were first observed in flexibacterial cultures by Lewin (1). They are cylinders, approximately 225 nm long and 33 nm in diameter, from which a "wick-like" process, 15 nm in diameter, may extend for various distances up to 1600 nm (1, 2). Rhaphidosomes from *Saprosira grandis* WH have been reported to contain RNA, the nucleotides of which were at least 85-percent 2'-O-methylated (2). Such a high level of O-methylation has not been reported for RNA from any other source. Particles having rhaphidosome morphology have been described from such diverse sources as *Archangium violaceum* (3), *Proteus mirabilis* (4), and *Actinomyces streptomycini* (5). However, only rhaphidosomes

from *S. grandis* WH have been isolated and chemically examined.

Seven strains of flexibacteria (6) were cultured in aerated carboys of enriched sea-water medium, and the rhaphidosomes were isolated by a reported (2) method. Ribonucleic acid was extracted by modified phenol procedures from rhaphidosomes (2) and from cells of *S. grandis* WH harvested by centrifugation during the early part of the growth curve (7). When the procedure used for extraction of cells was tested on isolated rhaphidosomes, it failed to extract that RNA. All RNA fractions were first chromatographed on columns (2.5 by 35 cm) of diethylaminoethyl-cellulose (DEAE-cellulose) (7).

Table 1. Products of hydrolysis of rhabidosome RNA (seven strains) for 18 hours at 35°C in 0.5N KOH. Abbreviations: O.D., optical density; ONT, oligonucleotides; MNT, mononucleotides; CMP, cytidine monophosphate; AMP, adenosine monophosphate; GMP, guanosine monophosphate; UMP, uridine monophosphate.

Strain	Percentage of O.D. at 260 nm		Base distribution of MNT released (mole %)			
	ONT	MNT	CMP	AMP	CMP	UMP
WH	97	3	55			45
Daw-2	93	7	52	3	7	38
Gol-12	75	25	36	25	22	17
Jl-4	88	12	50	14	9	27
Si-09	95	5	39	12	23	26
Eg-13	86	14	47			53
Lim-21	40	60	17	11	25	47

In the case of *S. grandis* RNA, the soluble and ribosomal fractions were combined and enzymically hydrolyzed for freeing of nucleosides; the methylated fraction of nucleosides was separated from the nonmethylated fraction by zone electrophoresis in borate buffer, and the two fractions were eluted with dilute HCl for absorption measurements at 260 nm (2).

The methylated fraction of nucleosides was too small for detection by these methods (less than 5 percent); thus the bulk of the soluble and ribosomal RNA from *S. grandis* cells was not highly methylated and was different in type from the RNA isolated from the rhabidosomes. Rhabidosome RNA fractions from DEAE-cellulose chromatography were dialyzed against distilled water, concentrated with a flash evaporator, and hydrolyzed in 0.5N KOH at 35°C for 18 hours. The hydrolyzates were neutralized in the cold with HClO<sub>4</sub>, and the KClO<sub>4</sub> that formed was removed by centrifugation at 0°C.

The fractions were then applied to fresh DEAE-cellulose columns (2.5 by 35 cm) which were eluted as before hydrolysis. Free nucleotides or dinucleotides, which were eluted early in the gradient, were applied to a Dowex-1-10X-formate column (1.2 by 30 cm). The Dowex-1 columns were eluted (8), and fractions were tested for absorption at 260 nm. Absorption spectra and orcinol (9) assays were routinely measured for all column fractions in which absorption at 260 nm was found. 2'-O-Methyl ribose gives approximately the same orcinol assay per mole as does ordinary ribose (9).

All seven rhabidosomes contained RNA that was always highly 2'-O-methylated (Table 1). The 2'-O-methyl group confers stability to alkaline hydrolysis only to the phosphodiester linkage at the 3'-position of the methyl-

ated nucleotide. Mononucleotides that are released must be nonmethylated themselves and must also be linked at the 5'-position to another nonmethylated nucleotide. The mononucleotides released from the RNA of the rhabidosomes varied in base distribution from strain to strain and were nonrandom. In some instances the nucleotides released were almost exclusively pyrimidine nucleotides. If a single methylated nucleotide is found within a nonmethylated portion of the chain, a dinucleotide will be released by alkaline hydrolysis. Only traces (less than 1 percent) of such dinucleotides were found in any of the seven rhabidosome RNA hydrolyzates.

I conclude that flexibacterial rhabidosomes constitute a class of particle that contains an RNA that is unusual in that it is highly but not completely 2'-O-methylated. The distribution of this methylation along the RNA chains and among the nucleotides of the chains is nonrandom and varies from strain to strain.

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#### References and Notes

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2. D. L. Correll and R. A. Lewin, *Can. J. Microbiol.* **10**, 63 (1964).
3. H. Reichenbach, *Arch. Mikrobiol.* **50**, 246 (1965).
4. W. Van Iterson, J. F. Hoeniger, E. N. Van Zanten, *J. Cell Biol.* **32**, 1 (1967).
5. Y. I. Rautenstein, S. B. Stefanov, E. I. Smirnova, L. N. Khadji-Murat, *Microbiologia* **30**, 860 (1966).
6. Flexibacterial cultures were obtained from R. A. Lewin, Marine Biology Department, Scripps Institution of Oceanography, La Jolla, Calif.
7. D. L. Correll and N. E. Tolbert, *Plant Physiol.* **37**, 627 (1962).
8. D. L. Correll, *Plant Cell Physiol.* **6**, 661 (1965).
9. ———, *Phytochemistry* **4**, 453 (1965).
10. Aided by Smithsonian Research Foundation grant 58-0001-3357. Publication by approval of the secretary, Smithsonian Institution.

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## Rupture Mechanism of a Liquid Film

Abstract. *The rupture mechanism of edge-supported liquid films appears to involve the viscous and drag energies as well as previously postulated kinetic and surface energies. Although details are obscure, the mechanism appears to involve a liquid-gathering process at the free edge, followed by fragmentation of this thickened edge into drops whose radii are approximately 50 times the film's original thickness.*

Little work has been reported concerning the processes occurring during the collapse of a liquid film. Dupre (1), who was the first to study the film-rupturing process, calculated the speed at which a liquid film ruptures by assuming that the surface energy of the film is completely converted into the kinetic energy of the liquid according to the relation

$$2 \nu S = \frac{1}{2} S \lambda \rho V^2 \quad (1)$$

where  $\nu$  is the surface tension of the film in dynes per centimeter,  $S$  is the total surface area of the film in square centimeters,  $\lambda$  is the film thickness in centimeters,  $\rho$  is the liquid density in grams per cubic centimeter, and  $V$  is the velocity of the retracting edge in centimeters per second. Rayleigh (2) and Devries (3) studied the rupturing of soap films formed on circular wire frames and measured rupture velocities of 350 to 1500 cm/sec. The work of Devries indicates that the rupture velocity remains constant during the entire process, which suggests that the phenomena quickly attain a steady state. Droplet formation during film rupture apparently was not observed by these investigators, and the film thicknesses were not reported; thus it is difficult to assess the suitability of Dupre's relation. It would appear, however, that this equation can only give an upper limit for the velocity of the free edge of the film, since such dissipative forces as drag and viscosity were neglected. While studying the bursting of bubbles at liquid-air interfaces, other investigators (4) noted the formation of a fine spray of drops at the instant of burst.

This report describes our investigation of the processes occurring in the vicinity of a free edge during film rupture. In our experiments we used flash (8  $\mu$ sec) photography, flash interferometry, and high speed (5000 frame/