THE BASE COMPOSITION OF RNA FROM NUCLEOLI FORMED AT TWO DIFFERENT ORGANIZERS IN CHIRONOMUS TENTANS

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In animal and plant cells the nucleolar RNA is thought to be templated by less than a percent of the genomic DNA, but still a quantity large enough to contain several cistrons (McConkey and Hopkins, 1964; Chipchase and Birnstiel, 1963; Perry et al., 1964). It is a matter of dispute whether the cistrons are located exclusively at the loci of nucleolar formation and the nucleolar RNA formed locally as believed by McMaster-Kaye (1960), Amano and Leblond (1960), Sirlin (1960), Sisken and Kinosita (1961), Perry et al. (1961) and Pelling (1964), or whether the nucleolar RNA is, partly or completely, synthesized at other parts of the genome (Goldstein and Micou, 1959; Woods, 1959; Rho and Bonner, 1961; Chipchase and Birnstiel, 1963). Irrespective of whether in some cases the latter alternative would be true or not, it has been shown clearly for Chironomus tentans that the nucleoli are synthesized locally (Pelling, 1964). In this case there are two pairs of nucleolar organizers, one at the second and one at the third chromosome. This type of distribution is in no way a general attribute of metazoan cells or even of Chironomus. The fact that the cistrons for nucleolar RNA formation are separated into two groups could, however, possibly reflect chemical differences between the DNA molecules and their products, differences that might not always express themselves

morphologically. The question whether there are any base compositional differences between the two nucleoli has been investigated by microelectrophoresis of RNA components from nucleoli individually isolated from salivary gland chromosomes.

Materials and Methods

Salivary glands removed from fourth instar larvae of Ch. tentans were placed in ethanol-acetic acid (3:1) for 5 min, followed by 50 % acetic acid for 1-2 min. A gland was then placed in a droplet of the 50 % acetic acid on a coverslip and the secretion removed with a watchmaker's forceps and a needle. The coverslip was placed, inverted, with the cells still wetted by acetic acid on top of an oil chamber and the chromosomes were isolated with two glass needles, maneuvered by the de Fonbrune micromanipulator. The swollen nucleoli could easily be disengaged from the chromosomes and nucleoli from the second and third chromosome were pooled separately in groups of 5-10 nucleoli. RNA was extracted with ribonuclease solution, hydrolyzed and analyzed by microelectrophoresis (Edström, 1964), and two or three analyses were run of each extract.

Results and Discussion

On account of the high nucleolar RNA concentration the RNA extracts are relatively clean and the analyses comparatively accurate. It was therefore possible to determine the mean values with moderate standard errors. In spite of this no significant difference was found between the two groups, each consisting of six animals, and the composition agreed well in the two cases (Table I).

Table I

Source of nucleolus	Moles of base in percent of the sum ± standard error of the mean			
	adenine	guanine	cytosine	uracil
Chromosome II	31.0±0.3	22.8±0.2	18.5±0.3	27.7±0.4
Chromosome III	30.8±0.4	22.2±0.3	18.8±0.6	28.3±0.5

As has been recorded earlier (Edström and Beermann, 1962) the base composition is unusual in the sense that it has a high content of adenine and uracil, although not as high as the content of adenine and thymine for the bulk of DNA. On the other hand it closely resembles the cytoplasmic RNA, in agreement with the view that nucleolar RNA, at least partly, is a precursor of ribosomal RNA (Perry 1962, 1963 and 1964; Franklin and Baltimore, 1962; Chipchase and Birnstiel, 1963; Brown and Gurdon, 1964; McConkey and Hopkins, 1964; Edström, 1965).

The results on base composition are negative in the sense that they do not exclude that there are differences too small to be detected or differences in other qualities. They do, however, point to a high degree of similarity, contrasting in this respect to the results of RNA base analyses of different chromosomal segments not involved in nucleolar RNA formation, between which marked and significant differences are found (Edström and Beermann, 1962). The present results are in harmony with the studies of Beermann (1960), demonstrating a complete functional equivalence between the organizers and even their parts in hybrids between Ch. tentans and Ch. pallidivittatus.

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