nodiffusion technique employed, however, is limited. With this technique, one can indicate only serological relationships but not similarity of proteins[14]. Even determining the degree of serological relationship between these 2 proteins by this technique has proved difficult in my hands because of the different types of reactions that I have obtained by varying the relative concentrations of antigen and antibody. It is therefore difficult to follow the kinetics of the transformation process with the immunodiffusion technique alone and I was thus prompted to study transformation simultaneously with a biochemical technique as well as by immunological methods. My results show that i-Ag D and G have the same mol.wt after reduction, but slightly different mol.wts without reduction (260,000 for serotype G and 240,000 for serotype D). These differences are quite small if one considers the total size of the proteins. Such a difference may be explained by a different behavior during migration of these proteins in the absence of reducing agent, which could be accounted for by a difference in their tertiary structure rather than their primary structure. One can imagine that one or several small peptides (glycopeptides?) may be added to the core, and these may change the mol.wt and the tertiary structure of these proteins, and thus the migration in unreduced gels would be different. These proteins thus may have many common polypeptide segments, but a few different segments which confer antigenic specificity.

I found that the new protein is more rapidly detected by electrophoresis than by immobilization tests. This result tends to support the hypothesis that proteins are newly formed in the cytoplasm and subsequently move to the cell surface, because the extraction procedure for electrophoresis may remove not only the proteins exposed on the cell surface, but also those within the cell or in the process of being externalized, therefore, electrophoretic detection would preceed detection by the in vivo immobilization tests. With the PAA technique, we have obtained a precipitate, detected by electrophoresis, only between homologous antigens and antibody. There is no discernible precipitate between heterologous antigen and antibody in the high mol.wt zone. This result shows indeed that i-AgG and i-AgD probably have completely different antigenic sites, but it gives us no information about the primary structure of the proteins. The absence of precipitation bands between heterologous antigen and antibody in the zone of the high mol.wt would indicate that the polypeptide common to serotypes G and D (detected by immunodiffusion) probably has a lower mol.wt than the i-Ag itself.

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Silver staining in *Drosophila melanogaster:* NOR behaviour in heteroploid cultured cells

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Summary. A heteroploid cell line of *Drosophila melanogaster* was cytologically analyzed by silver staining to investigate the in vitro behaviour of NORs. A single Ag-positive NOR was detected in all the metaphases observed, suggesting a possible suppression of excess ribosomal genes.

The silver staining technique devised by Goodpasture and Bloom[2] was used with success to visualize nucleolus organizer regions (NORs) in cells of different type and origin[3–7]. Cytological and biochemical studies have demonstrated that this stainability of chromosomal NORs depends on the activity of the ribosomal genes in the preceding interphase[8–11], and that silver binds to a single nucleolar protein[12] involved in the transcription or in the post-transcriptional processing of rRNA.

Recently this technique also proved to be effective in *Drosophila melanogaster* cells cultured in vitro, where only one active NOR was detectable in a diploid female karyotype[13].

In order to investigate the in vitro behaviour of NORs, a *Drosophila* cell line, characterized by structural and numerical variations, was analyzed by silver staining.

Materials and methods. The 10P102 line, derived from *D. melanogaster* T(Y;3)P102 stock, was established by Prof C. Halfer in our laboratory and maintained in D225 medium[15] supplemented with 20% fetal calf serum. This line was used because of its karyotypic characteristics, i.e. various rearrangements (differing from that of the parental stock), centric misdivision in some of the 2nd chromosomes and, above all, the presence of heteroploid chromosome numbers ranging from 14 to 23 (M = 17–20), as described previously[14]. The Y chromosome is not present in these cells. As for the X chromosomes, their number may be from 2 to 4 in a cell and one or more of them may be affected by structural changes such as a translocation involving the distal euchromatic portion or a marked increase in the heterochromatic portion (X_L = X long)[14].

Silver staining was carried out by a modification of the Ag-

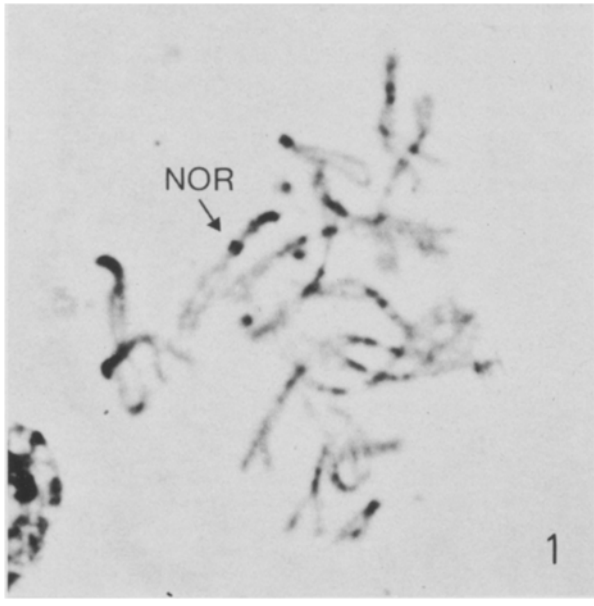

Fig. 1. A 10P102 metaphase (chromosome number = 19) showing an Ag-positive NOR on the X_L chromosome.

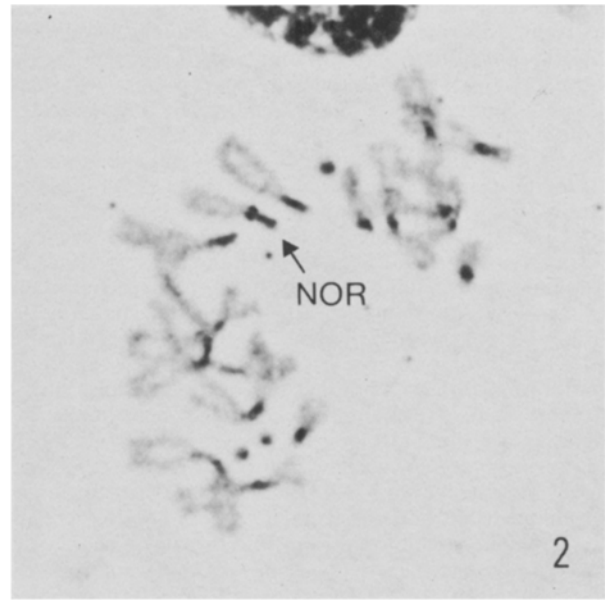

Fig. 2. A 10P102 metaphase (chromosome number = 20) showing an Ag-positive NOR on one of the X chromosomes.

AS technique devised by Goodpasture and Bloom[2]. First the slides were incubated in 50% silver nitrate at 55 °C for 4 h, then developed by adding 2 drops of AS solution (4 g $AgNO_3$ + 5 ml H_2O + 5 ml NH_4OH: pH 12–13) and 2 drops of 4% formaldehyde solution neutralized with sodium acetate crystals. As soon as the slides became deep yellow, they were rinsed in distilled water, air-dried and mounted in Euparal.

Results and discussion. A total of 223 metaphases was analyzed. In spite of the chromosome number heterogeneity of this line[14], all the Ag-positive cells (120) showed a homogeneous Ag-pattern, only 1 of the X chromosomes carrying silver grains at the NO specific site[16]. The presence of Ag-negative cells depends on differences in the intensity of staining in different preparations and in different areas of one slide.

Previous studies on a diploid XX_L cell line indicated the presence of active silver stained NORs only on the X_L chromosomes, suggesting a possible relation between NOR stainability and the X_L heterochromatin increase[13].

The present results indicate that silver deposited only on the X_L nucleolus organizer in those cells where this chromosome was present. Nevertheless, when there was no X_L chromosome in the karyotype, stained NORs appeared on normal Xs. In both cases, only 1 of the Xs in a cell carried an Ag-positive nucleolus organizer region. Therefore, in these polyploid conditions one NOR seems to take over the entire nucleolar activity of the cell, as if the transcription of NORs in excess were suppressed.

A similar NOR behaviour was observed in mouse-human somatic hybrid cells[8], where suppression of the transcriptional activity occurred in human chromosome NORs.

Curiously, many years ago Navaschin[17] had already observed that in F_1 hybrids of certain species of *Crepis* the NO of 1 of the parents was no longer visible.

The present report may also agree with the findings in neoplastic human cells[18], where the number of Ag-positive NORs was stable and comparable to that observed in control subjects, in spite of the significant increase in the number of D- and G-group chromosomes.

Regarding *Drosophila* cells, the data reported are not in disagreement with the hypothesis of a regulation of the number of ribosomal genes[19]

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