

Distribution of rRNA genes in breeds of domestic pig studied by non-radioactive *in situ* hybridization and selective silver-staining

CHM Mellink¹, AA Bosma¹, NA de Haan¹, J Wiegant²

¹ Faculty of Veterinary Medicine, Utrecht University,
Department of Cell Biology and Histology, PO Box 80.157, 3508 TD Utrecht;

² Faculty of Medicine, Leiden University, Department of Cytochemistry and Cytometry,
Leiden, The Netherlands

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INTRODUCTION

Radioactive *in situ* hybridization in the domestic pig has revealed that the rRNA genes are located either in the secondary constriction of chromosome 10 only (Miyake *et al*, 1988), or in the secondary constrictions of chromosomes 10 and 8 (Toga-Piquet *et al*, 1984; Vagner-Capodano *et al*, 1984).

Silver (Ag) staining has been proven to be a reliable technique for demonstrating nucleolar organizer regions (NORs) on chromosomes and to be indicative of active ribosomal RNA synthesis (Howell, 1982).

Variations in number and size of Ag-NORs have been observed in several species, including the pig (Czaker and Mayr, 1980, 1982; Mayr and Schleger, 1981; Vischnevskaya and Vsevodolov, 1986). According to these authors, silver deposits are largest on chromosomes 10, while they are smaller or absent on chromosomes 8.

In the present study, chromosomes of 6 different pig breeds were examined for: 1) the localization of the rDNA, using non-radioactive *in situ* hybridization, 2) Ag-NOR staining patterns, and 3) polymorphism for the amount of rDNA.

MATERIALS AND METHODS

Blood samples were collected from 40 animals belonging to the following breeds: *Dutch Landrace*, *Yorkshire*, *Norwegian Landrace*, *Finnish Landrace*, *Piétrain* and *Meishan*. Cultures of phytohemagglutinin (PHA)-stimulated peripheral blood lymphocytes were set up, and chromosome preparations made, following standard procedures.

In situ hybridization was carried out in 11 pigs, using a biotinylated 5.2 kb *Bgl*III-*Eco*RI human rDNA probe (Wachtler *et al.*, 1989). Silver-staining of NORs was performed in all 40 pigs, following the Ag-AS (ammoniacal-silver) technique of Goodpasture and Bloom (1975).

An attempt was made to quantify the amount of silver deposited on the NORs by means of image analysis. The area of the silver deposit was divided by the length of the chromosome.

RESULTS AND DISCUSSION

In situ hybridization revealed that the secondary constrictions of both chromosomes 10 and both chromosomes 8 were the sites of location of the rRNA genes in the breeds examined (table I) with the exception of the *Dutch Landrace* pig and one *Meishan* pig, which also had cells exhibiting only 3 fluorescent signals. However, in both cases, these exceptions can be explained by difficulties in detection due to the presence of a very small signal on one chromosome 8. In general, the size and intensity of the signals varied between chromosomes 10 and 8, between the two homologues of pair 10 and between the two homologues of pair 8. In most animals, the signals on chromosome 10 were largest, but in some animals (the *Yorkshire* pig and one *Piétrain* pig) chromosome 8 had a stronger signal. These observations suggest that differences exist in the amounts of rDNA present at the 4 nucleolar organizer regions.

Table I. Numbers and cellular distribution of *in situ* hybridization signals in 11 pigs of 6 breeds.

| Breed | n | 10/10 -/- | 10/10 8/- | 10/10 8/8 |
|---------------------------|----|--------------|--------------|--------------|
| <i>Dutch Landrace</i> | 45 | 0 | 21 | 24 |
| <i>Yorkshire</i> | 20 | 0 | 0 | 20 |
| <i>Norwegian Landrace</i> | 10 | 0 | 0 | 10 |
| <i>Finnish Landrace</i> | 20 | 0 | 1 | 19 |
| <i>Piétrain</i> | 20 | 0 | 0 | 20 |
| <i>Piétrain</i> | 20 | 0 | 1 | 19 |
| <i>Meishan</i> | 15 | 0 | 0 | 15 |
| <i>Meishan</i> | 15 | 0 | 5 | 10 |
| <i>Meishan</i> | 15 | 0 | 0 | 15 |
| <i>Meishan</i> | 20 | 0 | 0 | 20 |
| <i>Meishan</i> | 20 | 0 | 0 | 20 |

n = number of cells studied.

Silver staining revealed either 2, 3 or 4 Ag-NORs in the cells of the animals studied. The NORs of chromosomes 10 were consistently silver-positive, whereas the NORs of chromosomes 8 varied in staining. We concluded that the NORs of chromosomes 10 were transcriptionally active in all cells, whereas the NORs of chromosomes 8 were not or were active to a variable degree. Table II presents mean numbers of Ag-NORs for each breed. The lowest mean numbers were found in the *Dutch*, *Norwegian* and *Finnish Landraces*. Moderate mean numbers were observed in the *Yorkshire* and *Piétrain* breeds. An extremely high number of Ag-NORs was scored in the *Meishan* breed. The results of this study include higher mean numbers of Ag-NORs than have been found for certain other breeds: *Austrian Landrace* 2.17, *Deutsch Edelschwein* 2.24, *Belgian Landrace* 2.38 (Czaker and Mayr, 1982). However, Czaker and Mayr (1982), and Vischnevskaya and Vsevodolov (1986) found similar mean Ag-NOR values for the *Piétrain* breed (2.54), and for the *Large White* and *Duroc* breeds (2.53 and 2.75, respectively). The ranges given in table II demonstrate that, within the breeds investigated in this study (with the exception of the *Meishan* breed), considerable individual variability exists with regard to the Ag-NOR number.

Table II. Mean numbers of Ag-NORs in 6 pig breeds.

| <i>Breed</i> | <i>n</i> | <i>Range</i> | <i>X ± SD</i> |
|---------------------------|----------|--------------|---------------|
| <i>Dutch Landrace</i> | 10 | 2.04–3.04 | 2.51 ± 0.37 |
| <i>Yorkshire</i> | 5 | 2.13–3.73 | 2.77 ± 0.73 |
| <i>Norwegian Landrace</i> | 7 | 2.04–2.88 | 2.32 ± 0.32 |
| <i>Finnish Landrace</i> | 7 | 2.00–3.12 | 2.54 ± 0.51 |
| <i>Piétrain</i> | 6 | 2.20–4.00 | 3.06 ± 0.78 |
| <i>Meishan</i> | 5 | 3.94–4.00 | 3.97 ± 0.03 |

n = number of animals studied.

Apart from the variation in Ag-NOR number, variation in the amount of silver deposited was observed. In most cells, the largest amounts of silver were present on chromosomes 10. Chromosomes 8 usually showed much smaller Ag-NORs or no (detectable) silver deposit at all. Also, in some animals, consistent differences in Ag-NOR size between homologous chromosomes were observed. Figure 1 shows a metaphase spread with Ag-NORs on chromosomes 10 (distinct dimorphism) and 8 (slight dimorphism).

The results of quantification by image analysis of the amounts of silver deposited on the NORs of the 5 *Meishan* pigs are presented in figure 2. Some of these animals showed relatively little polymorphism for the amount of silver on their NORs, whereas the others showed a more marked polymorphism in this respect. Again, the Ag-NORs on chromosomes 10 were usually the largest. The metaphase spread shown in figure 1 came from the animal second from left in figure 2. These quantification studies will be continued.

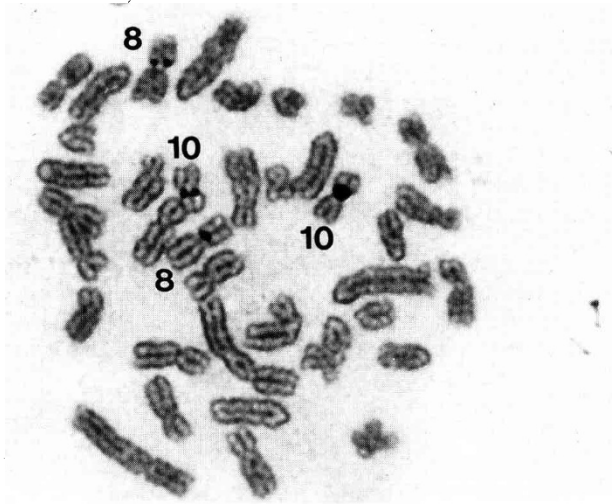


Fig 1. Silver-stained metaphase spread from a *Meishan* pig, showing polymorphism of the Ag-NORs.

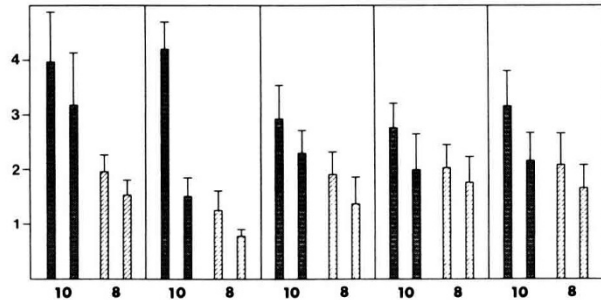


Fig 2. Histograms showing the relative size of the Ag-NORs in 5 *Meishan* pigs (mean values based on 5 cells).

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