Genet Sel Evol (1990) 22, 273-277

Original article

Nucleolus organizer regions, types of association and identification of carrier chromosomes in domestic sheep

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(Received 20 November 1989; accepted 15 May 1990)

Summary – The frequency of chromosomes carrying nucleolus organizer regions (NOR-positive) and the percentage of association between these chromosomes were determined in sheep metaphase spreads. The sequential R-NOR banding technique permitted identification of the chromosome regions involved as the telomeric ends of chromosomes 1 (1p), 2 (2q), 3 (2q), 4 and 25.

nucleolus organizer regions / sequential R-NOR banding / sheep

Résumé – Régions de l'organisateur nucléolaire, types d'associations et d'identification des chromosomes porteurs chez le mouton. *La fréquence de chromosomes porteurs de la région d'organisation nucléolaire (NOR) et le pourcentage d'association entre chromosomes NOR-positifs ont été étudiés chez le mouton. La méthode de marquage séquentiel R-NOR a permis d'identifier comme régions chromosomiques impliquées, les télomères des chromosomes 1 (1p), 2 (2q), 3 (3q), 4 et 25.*

régions de l'organisateur nucléolaire / bandes séquentielles R-NOR / mouton

INTRODUCTION

The silver-staining technique developed by Goodpasture and Bloom (1975) permitted the differential staining of chromosome proteins from particular regions of the chromosome, the nucleolus organizer regions. Miller *et al* (1976) showed that only the active NORs were stained with silver in human-mouse somatic cell hybrids.

In domestic sheep, the NORs are located in the telomeric regions of 5 pairs of chromosomes. In this paper, the frequency distribution of these chromosomes is determined, and the NOR-carrier chromosomes are identified using the R-NOR sequential banding technique.

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MATERIALS AND METHODS

Blood samples were collected from the jugular vein of 12 Spanish Merino sheep and cultured with autologous plasma as a modification of the original whole blood culture method described by De Grouchy *et al* (1964). The metaphase spreads were banding using the R-NOR banding technique developed by Di Berardino *et al* (1985). The number of metaphase spreads examined per animal varied from 10-30, and for each spread, the number of NOR-positive chromosomes was recorded. The preparations were examined under UV light and micrographs of the best R-banded metaphases were taken using Kodalith film and printed on Valca No 2 paper.

RESULTS AND DISCUSSION

The R-NOR sequential banding technique permitted identification of the positive Ag-NOR chromosomes of sheep (fig 1). The nucleolus organizer regions are located on the telomeric ends of 2 acrocentric (4 and 25) and 3 metacentric chromosomes (1p, 2q, and 3q).

The frequency of NOR positive chromosomes varied between individuals (table I). The number of NORs per metaphase varied from 5-7.71, with an average of 6.31 NORs/metaphase. These results are similar to those reported in cattle (Mayr *et al*, 1987), and in goats (Moreno-Millán and Rodero, 1988a), but appear lower than those reported in sheep (8 NORs/metaphase) by Henderson and Bruere (1977). Individual variations in the number of chromosomes showing active NORs have already been reported in humans, pigs, cattle, horses and goats (Goodpasture *et al*, 1976; Ray and Pearson, 1979; Stefanova, 1983; Mayr *et al*, 1987; Kopp *et al*, 1988; Moreno-Millán and Rodero, 1988a).

Table I. Mean number and standard error (SE) of Ag-NORs per metaphase in 12 sheep.

Animal No	*No Metaphases*	*Mean No Ag-NOR*	SE
1	15	5.66	0.43
2	12	5.17	0.76
3	26	6.69	0.34
4	17	5.00	0.31
5	16	7.19	0.29
6	23	7.44	0.24
7	17	6.70	0.44
8	18	5.83	0.46
9	22	5.55	0.42
10	26	5.42	0.30
11	30	7.37	0.31
12	24	7.71	0.36

Variations in the number of chromosomes showing positive NOR-staining occurred also in the metaphase spreads from the same animal (table I). In some metaphase spreads, both homologous chromosomes were stained, whereas in others

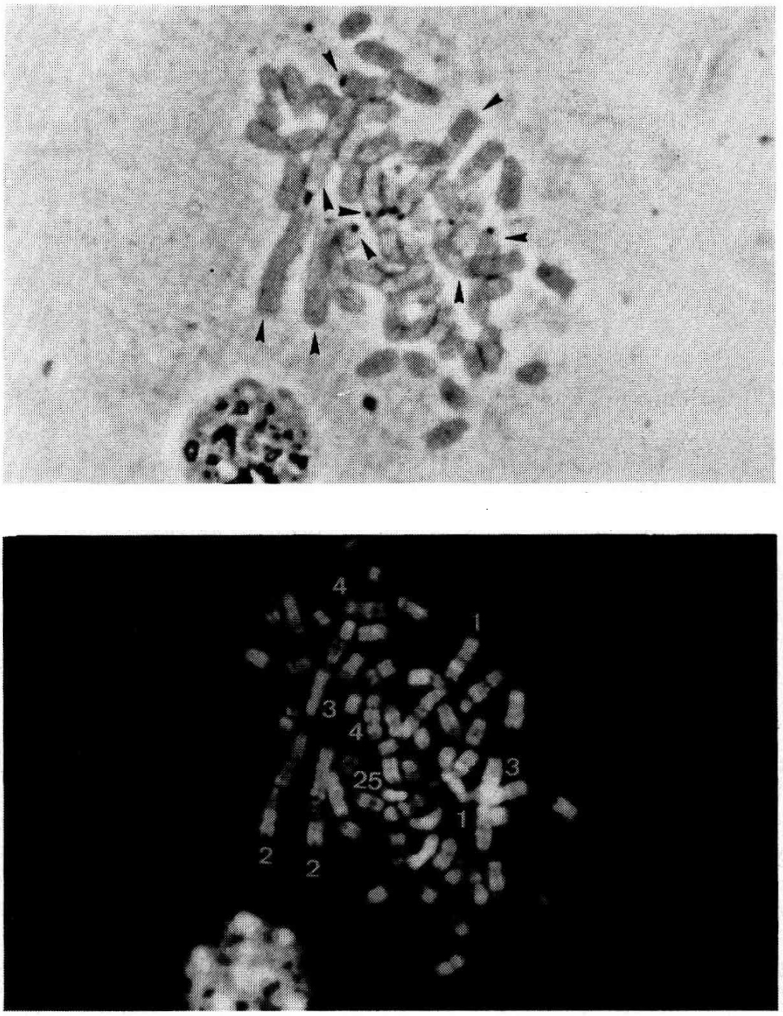

Fig 1. Sequential R-NOR banding in sheep: a) The NOR-active carrying chromosomes are indicated by arrows. b) R-banding of the same metaphase.

only one chromosome of a given pair was NOR-positive. This variability has already been observed in cattle, goats, horses and humans (Mikelsaar *et al*, 1977; Mayr *et al*, 1987; Kopp *et al*, 1988; Moreno-Millán and Rodero, 1988a).

The frequency of total NORs per metaphase showed a trimodal distribution of 4, 6 and 8 positive NORs per cell (table II). This suggests the presence of different peripheral blood lymphocyte (PBL) populations, with a selective advantage given to the 3 types noted in culture. Similar results were obtained in cattle (Mayr *et al*,

Table II. Frequency of Ag-NORs per metaphase.

Ag-NORs No	*No metaphases*
2	2
3	12
4	41
5	21
6	55
7	32
8	61
9	12
10	10

Table III. Distribution of the chromosomes involved in associations ($\chi^2, P < 0.05$).

Chromosomes No	*Obs*	*Exp*	χ^2
1	29	31.2	0.16
2	13	31.2	10.62*
3	17	31.2	6.46
4	57	31.2	21.33***
25	40	31.2	2.48
Total	156	156	

1987), but as noted in this study, this trimodal distribution did not occur in specific animals.

The average rate of association between Ag-NOR-positive chromosomes was 0.30 per metaphase, and could be divided into associations between 2 or 3 chromosomes (average rates of 0.28 and 0.20, respectively). This association rate is lower than that observed in goats (Moreno-Millán and Rodero, 1988b). Preferential association between certain chromosomes was noted (table III) whereas random association had been noted by Henderson and Bruere (1977). In cattle, Mayr and Schleger (1982) observed random association of chromosomes in only 1 of the 3 breeds investigated.

Associations between chromosomes appears to reflect the dynamic formation of the nucleolus (Henderson and Bruere, 1977). However, an increase in the amount of rDNA in the chromosomes seems to be correlated with an increased frequency of association, resulting also in a greater activity and increased silver staining (Henderson *et al*, 1973; Warburton *et al*, 1976; Millet *et al*, 1977). These aspects are currently being investigated, in association with studies on the transmission patterns of NOR frequency distribution in sheep, to give an overall picture of NORs in sheep karyotype.

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