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Mobile Nucleolus Organizing Regions (NORs) in *Allium* (*Liliaceae* s. lat.)? — Inferences from the Specifity of Silver Staining

By

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Key Words: Angiosperms, *Liliaceae, Alliaceae, Allium cepa, A. fistulosum, A. × proliferum.*—Nucleolus organizing regions (NORs), silver staining, ribosomal DNA, movable genetic elements.

Abstract: NORs and interphase nucleoli have been silver stained in *Allium cepa, A. fistulosum,* reciprocal crosses between both species, and in different strains of top onions which originated from hybridization between *A. cepa* and *A. fistulosum.* The variability observed in size, number, and position of active NORs and correspondingly in number (and size) of interphase nucleoli is at least in part strain-specific. These data are taken to indicate that NORs in *Allium* behave like movable genetic elements.—With respect to the staining specifity of silver nitrate, it was found that $AgNO_3$ labels (1) nucleoli, (2) NORs (i.e., actively transcribed "ribosomal genes") inside the achromatic secondary constrictions, and (3) sometimes (but less pronounced) centromeres; Giemsa banding labels heterochromatin surrounding the NOR but not the nucleolus organizing secondary constriction.

Movable genetic elements are well known in prokaryotes (IS elements, transposons, and bacteriophage Mu) as well as certain eukaryotes (for instance mating type genes of yeast, mobile dispersed genetic elements of *Drosophila,* control elements of maize, retroviruses of birds and mammals). For a comprehensive survey see Cold Spring Habor Symp. Quant. Biol. **45** (1981).

Additionally, so called "orphons", i.e., dispersed solitary genes derived from tandem multigene families (histone genes and ribosomal genes), have been found in sea urchin, *Drosophila,* and yeast, respectively (CHILDS & al. 1981). Ribosomal DNA sequences frequently

recombine between homologous, but also nonhomologous sites (see discussion). It is to be expected that the list of examples of mobile genetic elements will become much longer in future. In this paper arguments will be presented which suggest that active NORs in the genus *Allium* are mobile. This is borne out by the variability of number, size, and position of NORs and by the number and size of interphase nucleoli in different top onion strains[1], in the progenies from reciprocal crosses between *A. cepa* and *A. fistulosum* and sometimes even in the parental species.

Additionally, it will be demonstrated that the Giemsa banding technique and the silver technique stain different regions with respect to NORs and NOR-associated heterochromatin.

Material and Methods

Materials: Root tip meristems from bulbs, bulbils, and germinated seeds, respectively, of *A. fistulosum* (All 98)[2], *A. cepa*, of the cloned progeny from reciprocal crosses between both species (obtained by embryo culture in Olomouc, Czechoslovakia, see DOLEŽEL & al. 1980) and of five different strains of top onions (15, 80, All 228, 124, 437) have been investigated. For demonstration of N-banding, reconstructed karyotypes of *Vicia faba* have been used.

Methods: Giemsa banding according to SCHWARZACHER & al. (1980) and silver staining of NORs according to HOWELL & BLACK (1980) were used as described by SCHUBERT & al. (1983). The N-banding pattern of *Vicia faba* chromosomes was found during *in situ* hybridization experiments subsequent to the following procedure:

Digestion of fixed root tip meristems in pectinase (Sigma, 1%, pH 4.7, 1–2 hours at 37 °C); squashing in 45% acetic acid (dry-ice method); then: 45 min RNase (100 µg/ml) 20 °C; 30 min 2 × SSC, 65 °C; 25 min 0,2 n HCl, 37 °C; 10 min 70% ethanol; 10 min 96% ethanol, air-drying; 15 h 3 × SSC/50% formamide, 40 °C; 24 h 2 × SSC, 4 °C; 4 h 20 × SSC, 30 °C; 10 min 70% ethanol + 0.3 M ammonium acetate; 10 min 96% ethanol + 0.3 M ammonium acetate; air-drying; dipping in emulsion (ORWO K 6); 14 days storage at 4 °C; developing of film layer; 7 min staining in Giemsa (Merck) 1 : 25 pH 7.

Results

Normal and Deviating Number, Size, and Position of Active NORs and Nucleoli in Different Strains of *Allium*. *A. cepa* and *A. fistulosum* normally contain one pair of satellite chromosomes, each with an active

[1] In the accompanying paper (SCHUBERT & al. 1983) the hybridogenic origin of top onions [*Allium* × *proliferum* (MOENCH) SCHRAD.] from *A. fistulosum* L. and *A. cepa* L. was substantiated by application of Giemsa banding and silver staining of NORs.

[2] Strain numbers according to the Gatersleben gene bank collection, All = *Allium*.

Table 1. Number of satellites (Sat), number of active NORs, number of interphases scored (n), and percentages of these with 1,2,3, or more nucleoli in different species or strains (for the relative size of the nucleoli see footnotes)

Species, strains	Sat	NORs	n	Nucleoli			
				1	2	3	> 3
Allium cepa	2	2	2000	37.6	62.4[1]	—	—
A. fistulosum	2	2	2000	51.25	48.55[2]	0.2	—
A. fistulosum All 98	2	1	1000	94.2	5.7[3]	0.1	
A. cepa × *A. fistulosum*	2	2	1000	55.4	44.6[4]	—	—
A. fistulosum × *A. cepa*	1	2	1000	43.3	51.7[5]	4.3	0.5
Top onion strains: 15	1	1	1000	100.0	—	—	—
80	1	1	2000	99.6	0.4[3]	—	—
All 228	1	1	1000	97.8	2.0[3]	0.2	—
124	0	2–3	1000	34.9	61.0[6]	4.0	0.1
437	0	1–4	1000	68.0	25.0[7]	6.5	0.6

[1] Nucleoli of equal size; [2] proportion of nucleoli unequal in size varies between 4.4 and 98% in dependence on NOR extension; [3] second and third nucleoli are micronucleoli; [4] only 2.2% of interphases show nucleoli of unequal size; [5] in about half of the cases nucleoli of unequal size; [6] only 40 out of 610 interphases show nucleoli clearly unequal in size; third and fourth nucleoli always smaller; [7] one large and one or more small nucleoli.

NOR (large in *A. fistulosum* and smaller in *A. cepa*) at the secondary constriction (HIZUME & al. 1980, SCHUBERT & al. 1983). The corresponding interphases show one nucleolus or two nucleoli of equal size (see Table 1). Occasional deviations from this pattern may be observed: In *A. cepa* (cv. MAKOER) individuals with three nucleoli in some interphases were found (Fig. 4*b*). In *A. fistulosum* strain All 98, both homologous satellite chromosomes exhibit, after Feulgen staining, different distances of satellites from the short arms. In Giemsa banded metaphases, the proximal and distal heterochromatin blocks are so close to each other that the secondary constriction is no longer detectable in one of the homologues while it is well pronounced in the other (see SCHUBERT & al. 1983). This is not due to the squashing procedure, since by silver staining in many individuals only one homologous satellite chromosome shows an active NOR (see Fig. 1*a*), and interphase cells contain only one nucleolus, seldomly accompanied by a micronucleolus

(Table 1). In all probability, most (or all) rDNA sequences are deleted in one of the satellite chromosomes.

Recently HIZUME & al. (1980), after sequential staining first with Giemsa and then with silver nitrate, found in metaphases of *A. sativum* with three (!) active NORs, that the distances of the satellites from the short arm exactly correspond to the size of silver stained NORs.

In the progeny of crosses *A. cepa* × *A. fistulosum*, both satellite chromosomes revealed NORs corresponding to those of the parental species. In some individuals, the *A. cepa* NOR was much larger than in the parental species (see Fig. 1 *c*).

All cloned individuals from a cross between *A. fistulosum* × *A. cepa* showed a normal NOR at the satellite chromosome from *A. fistulosum;* the corresponding chromosome from *A. cepa* revealed however neither a satellite nor NOR. While the satellite of the original *A. cepa* satellite chromosome could not be detected elsewhere in the hybrid chromosome complement by Giemsa banding, an active NOR (smaller than that of *A. fistulosum*) was always found, by silver staining, in the telomeric position of a small nearly metacentric chromosome (see Fig. 1 *d*). Although this chromosome could not be individualized exactly after silver staining, it might belong to the chromosome complement of *A. fistulosum* since it is one of the shortest chromosomes (position 13–14 in most metaphases) and normally only the shortest chromosome pair of *A. cepa* may overlap in size with the two longest chromosome pairs of *A. fistulosum* as evident from Giemsa banding (SCHUBERT & al. 1983). Interphases of these hybrids often contain two nucleoli which are different in size and sometimes accompanied by a micronucleolus (see Fig. 4 *a*, Table 1).

The karyotypes of the top onion strains investigated may be distinguished by the morphology of the satellite chromosomes derived from *A. fistulosum* and *A. cepa*, respectively. Strains 15, 80, and All 228 show a satellite chromosome derived from *A. fistulosum* with a rather large satellite. The satellite of the original *A. cepa* satellite chromosome is deleted. In strains 124 and 437, the satellites of the corresponding chromosomes from both ancestor species got lost.

In top onions with a morphologically complete *A. fistulosum* satellite chromosome (strains 15, 80, All 228), this chromosome exhibits the only and under these circumstances very large NOR of the genome (Fig. 2 *a*). Interphase cells contain only one nucleolus and only very seldomly an additional micronucleolus (see Table 1).

Top onion strains (124, 437) without any true satellite always show a large NOR located at the end of the short arm of the original *A. fistulosum* satellite chromosome (Fig. 2*b*, 3). The original *A. cepa* satellite chromosome frequently (strain 124) or seldomly (strain 437) shows an

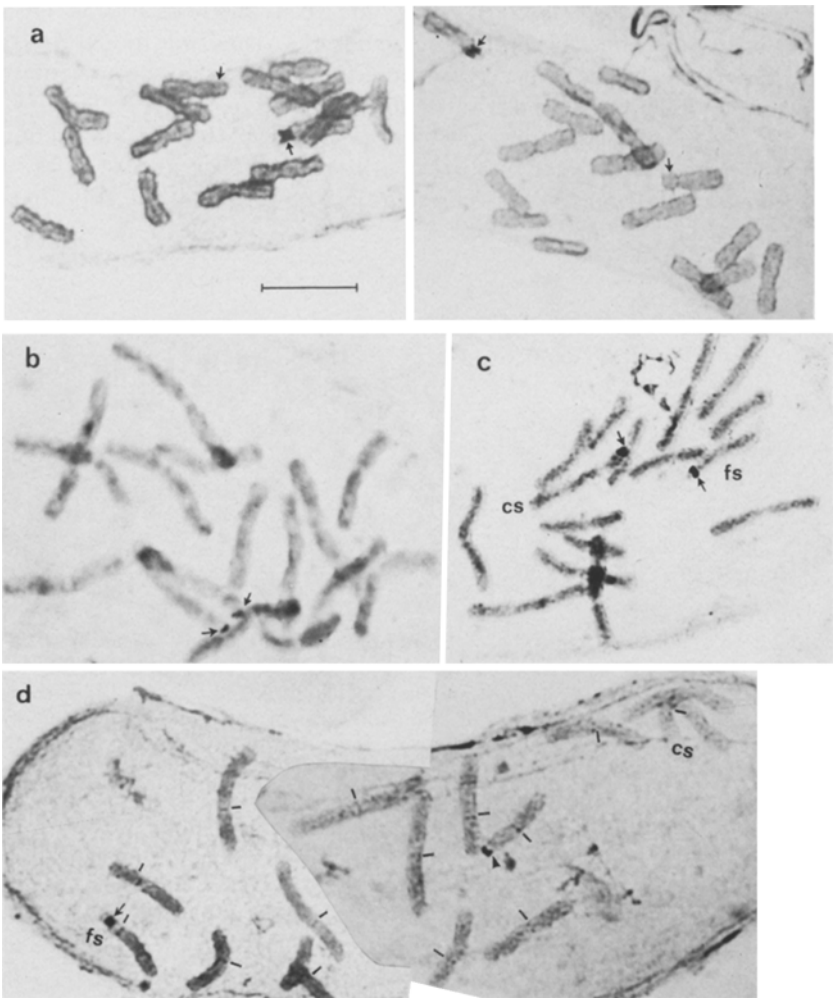

Fig. 1. Silver-stained metaphase chromosomes of *a* two cells of *Allium fistulosum*, strain All 98 (note the expression of NOR in only one homologue of the satellite chromosome pair: arrows); *b Allium cepa* cv. Australian Brown = All 27, the original satellite chromosomes are without satellites (NORs labelled by arrows); *c A. cepa* × *A. fistulosum*, NOR of the *A. cepa* satellite chromosome (cs) comparatively large—similar to that of *A. fistulosum* (fs) (arrows); *d A. fistulosum* × *A. cepa*, *A. cepa* NOR not at the former *A. cepa* satellite chromosome (cs) but at a small metacentric non-satellite chromosome (probably from the *A. fistulosum* chromosome complement, see arrowhead; also note the clearly labelled centromeric dots, indicated by small bars). The bar represents 10 μm

active NOR at the telomere of the short arm. In these strains, both NORs are sometimes fused (Fig. 3 *a*). One or two small additional NORs at the telomeres of other (non-satellite) chromosomes occasionally occurred (see Fig. 3) in both these strains.

If interphases of these strains have two nucleoli, they are (depending on the strain investigated) of different (in strain 437) or, in most cases, of equal size (in strain 124). If cells contain 3 or 4 nucleoli the additional

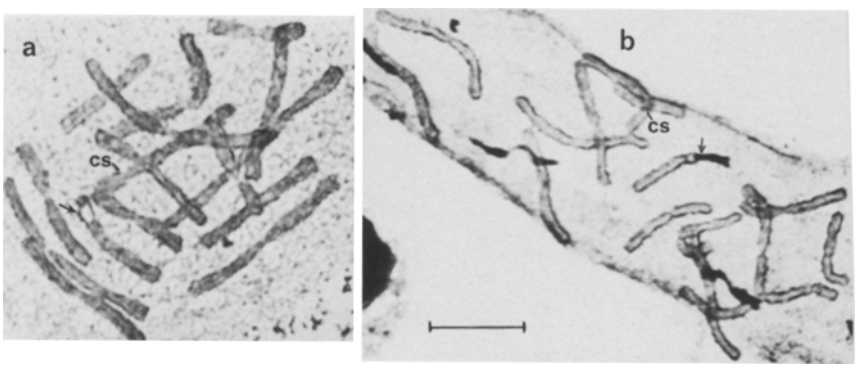

Fig. 2. Silver-stained metaphase chromosomes of top onions. *a* strain All 228 (note NOR only at the *A. fistulosum* satellite chromosome: arrow), while the former *A. cepa* satellite chromosome (cs) is without NOR; *b* strain 437 without any satellite, the only NOR is at the short arm of the former *A. fistulosum* satellite chromosome (arrow). Bar = 10 µm

nucleoli are always very small and their size corresponds to the less pronounced expression of the additional NORs at metaphase chromosomes. (Figs. 4 *c, d* and Table 1).

In one individual of strain 437, for instance, 15 (46.9%) of 32 metaphases had only one active NOR (at the former *A. fistulosum* satellite chromosome). In 11 (34.4%) metaphases, two NORs (the second in most cases at the former *A. cepa* satellite chromosome) were found. 3 metaphases showed 3, and 3 showed 4 NORs (each 9.4%). The differences between these values and the percentage of interphases with one or more nucleoli (see Table 1) may be explained as follows:

1. There may be some deviation between different individuals;

2. Due to nucleolar fusion at late interphase more cells than expected from the number of metaphases with only one NOR show only one nucleolus.

3. Some interphases with more (3 or 4) nucleoli remain undetected since these small nucleoli may be covered by the larger ones due to the squashing procedure.

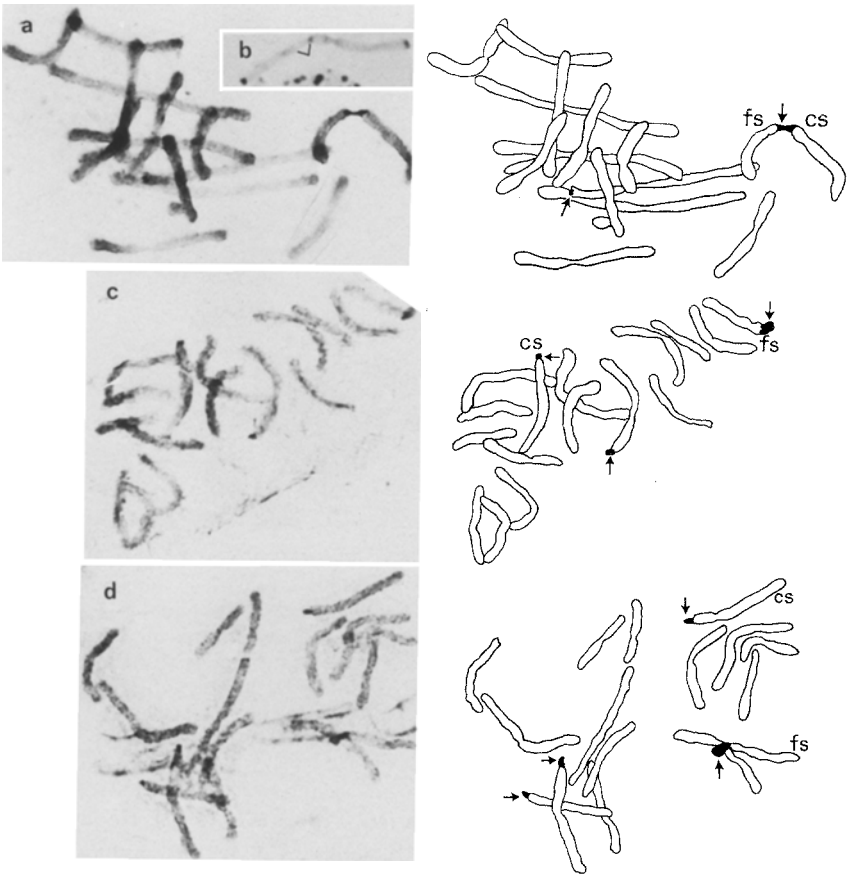

Fig. 3. Silver-stained metaphase chromosomes of top onions, photographs on the left, schemes on the right. *a* strain 124, three NORs (arrows), note the fusion of NORs from the former satellite chromosomes of *A. cepa* (cs) and *A. fistulosum* (fs); *b* NOR fusions between the former satellite chromosomes of *A. cepa* and *A. fistulosum* after Giemsa banding; *c* strain 437 metaphase with 3 NORs (arrows), at the former satellite chromosomes (cs and fs) and another *A. cepa* chromosome; *d* strain 437, metaphase with 4 NORs (arrows) at the former satellite chromosomes (cs and fs), at a non-satellite chromosome of *A. cepa* (vertical), and a non-satellite chromosome probably of *A. fistulosum* (horizontal)

Labelling of Centromeres After Silver Staining. Additional to the NORs—though distinctly less intensively—centromeres occasionally become positively stained with $AgNO_3$ (see Fig. 1 *d*). Such silver stained centromeric dots have already been reported for a number of other species: Mouse (Goodpasture & Bloom 1975, Miller & al. 1976), man

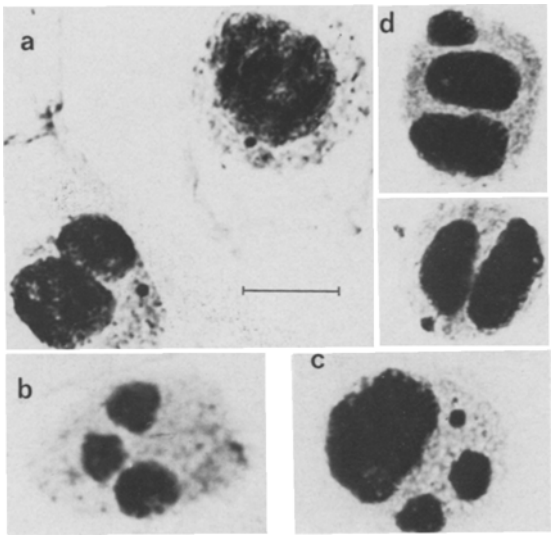

Fig. 4. Silver-stained interphase nucleoli. *a A. fistulosum* × *A. cepa;* left: two nucleoli of different size plus a micronucleolus, right: one nucleolus plus micronucleolus; *b A. cepa* cv. "Makoer", three nucleoli about equal in size; *c* top onion strain 437, one large, two smaller and one very small nucleoli; *d* top onion strain 124, above: two nucleoli of similar size and a smaller third nucleolus; below: two nucleoli somewhat different in size plus a micronucleolus. Bar = 10 µm

(DENTON & al. 1977), Indian muntjak (VED BRAT & al. 1979, BROWN and LOUGHMAN 1980), Chinese hamster (KAISERMAN & BURKHOLDER 1980), frog (BIRSTEIN 1981), *Odontophrynus occidentalis* (RUIZ & al. 1981). They may also occur in *Vicia faba* (SCHUBERT unpublished).

Discussion

Arguments for the Mobile Character of rDNA. A high frequency of recombination between rDNA sites of satellite chromosomes sometimes resulting in unequal exchange of material and even transpositions of these genes to non-satellite chromosomes seems to exist in genus *Allium*. This is indicated by the following observations:

1. Different size of silver stained regions, up to the disappearance of the NOR in one of the homologues (Fig. 1 *a*). In satellite chromosomes of *A. fistulosum* strain All 98, this occurs parallel to the different size of Feulgen-negative regions and the number (and size) of interphase nucleoli (Table 1).

2. The unusual large size of the *A. cepa* NOR (comparable to that of *A. fistulosum*) in some individuals of *A. cepa* × *A. fistulosum* hybrids (Fig. 1 *c*). In these cases, interphase nucleoli are of similar size, while in *A. fistulosum* × *A. cepa* hybrids with different size of both NORs the nucleoli are often of unequal size (Fig. 4 *a*, Table 1).

3. The frequently observed very large size of the NOR in the *A. fistulosum* satellite chromosome of top onions with only one detectable NOR (strains 15, 80, All 228), as compared to the size of the NORs of *A. cepa* and *A. fistulosum*, respectively. The larger single NOR of top onions probably consists of the rDNA sequences originally located in separate satellite chromosomes of *A. cepa* and *A. fistulosum*.

The dot-like micronucleoli sometimes occurring in top onion strains 15, 80, All 228 might indicate that remnants of rDNA active in transcription at the original position of the *A. cepa* satellite chromosome [and/or at other site(s)] still exist but escape detection by $AgNO_3$ since their copy number is too small for resolution in metaphase chromosomes.

4. Actively transcribed rDNA, even in a non-satellite chromosome present in *A. fistulosum* × *A. cepa* hybrids; concomitantly, the former *A. cepa* satellite chromosome is devoid of an active NOR (Fig. 1 *d*).

5. Additional NORs (to those occurring in the original satellite chromosomes) in some cells of top onion strains 124 and 437 at telomeric positions of non-satellite chromosomes (Fig. 3).

6. Fusions of silver stained NORs of both satellite chromosomes as frequently observed in metaphases of progenies from *A. cepa* × *A. fistulosum* crosses and of top onion strains 124 (Fig. 3 *a*) and 437. This has also been observed in Giemsa-banded metaphases (Fig. 3 *b*). The satellite assoziation of NOR-bearing mammalian chromosomes probably represents the same phenomenon and is also interpreted to facilitate recombinative exchanges between different rDNA sites (see for instance ARNHEIM & al. 1980).

Recombination between homologous and nonhomologous rDNA sites which may result in asymmetric exchanges (by unequal crossover) of these tandemly arranged repeated DNA sequences have been found in various organisms by different methods: in *Drosophila melanogaster* (TARTOF 1974), in yeast (SZOSTACK & WU 1980, ZAMB & PETERS 1981), in man and other primates (ARNHEIM & al. 1980), and in wheat (MILLER & al. 1980).

The different patterns of silver staining observed in the described strains of *Allium* species probably reflect, at microscopic level, aasymmetric recombination between the known sites of rDNA sequences in the satellite chromosomes, as well as rDNA transposition to new chromosomal sites. Indications of rDNA transposition were

obtained in *A. cepa*, in crosses *A. cepa* × *A. fistulosum* (in *A. sativum*, see HIZUME & al. 1980) and in satellite-less top onion strains 124 and 437, and suggest that rDNA of genus *Allium* may behave like a transposable genetic element. Recently, SATO (1981) found four individuals of *A. cepa* with 1, 2, 3, and 4 NORs, respectively, positioned either in the satellite chromosomes or in the smallest, nearly metacentric pair of chromosomes. This variability is assumed by SATO to be due to translocations or deletions but does not contradict the present interpretation that NORs are mobile in *Allium*.

That differences in extension of $AgNO_3$ stained NORs are not only due to differences in transcriptional activity, but also to different amount of rDNA, is indicated by quantitative comparison of silver stained regions and of number of silver grains after *in situ* hybridization with radioactively labelled rRNA between different rDNA sites in human (WARBURTON & HENDERSON 1979) and Chinese hamster metaphases (JHANWAR & al. 1981) and by parallel differences in extension of secondary constriction after Feulgen staining as well as after Giemsa banding. That NORs of non-satellite chromosomes are no artefacts is evident from the parallel occurrence of corresponding nucleoli at interphase.

Nucleolar dominance in the sense of NAVASHIN's (1927, 1928) "differential amphiplasty" may not be the reason for variability of NOR expression in top onions, since on the one hand in the progeny of crosses *A. cepa* × *A. fistulosum* and in many cells of top onion strain 124 both original NORs are active and on the other even in *A. fistulosum* one of the homologous NORs may be supressed.

The only alternative but less convincing interpretation of the data reported here is to postulate the existence of many rDNA sites (additional to those at secondary constrictions of satellite chromosomes) which are normally "silent" in *A. fistulosum* and *A. cepa* and may become differentially activated under "appropriate conditions" after species hybridization. According to this hypothesis, the "normal" rDNA site of *A. cepa* should become inactivated either without activation of another site (top onion strains 15, 80, All 228) or parallel to the activation of another site (*A. fistulosum* × *A. cepa*), or remains active inspite of activation of other sites (top onion strains 124 and 437). This interpretation was used by VERMA & RAINA (1981) to explain their finding of supernumerary nucleoli in pollen mother cells of *Crotalaria agatiflora*: "The change in the regulatory system of the cell caused by hybridity results in activation of latent nucleolar organizers although their overall presence in the genome is not due to hybridity." No evidence for this statement has however been presented. Transposition of rDNA to new chromosomal sites prior to the organization of

additional nucleoli may also explain the observation of supernumerary nucleoli. A clearcut decision between these two interpretations should become possible after *in situ* hybridization with radioactively labelled rRNA. If differential activation and inactivation of many rDNA sites in fact exist, *in situ* hybridization should regulary reveal rDNA sites not only at the secondary constrictions of the corresponding satellite chromosomes but also at least at one or two other chromosomal regions in the genomes of top onions, *A. cepa*, and *A. fistulosum*.

Interpretation of the data reported here by rDNA transposition gains support from an observation by ILYIN & al. (1978). These authors occasionally found additional nucleolus-like structures of different size at chromosomal sites different from that usually organizing the nucleolus in *Drosophila* polytene chromosomes. Only when a nucleolus-like structure was observed these sites became labelled by *in situ* hybridization with rRNA. Since transpositions of transcribed genes were always found at sites of intercalary heterochromatin, ILYIN & al. (1978) speculated that "intercalary heterochromatin regions are organized in such a way that certain genes can be easily inserted into them and can be easily lost as well".

In this connection it should be mentioned that the unambiguous additional NORs were found at telomeric positions which are known, from Giemsa and other banding techniques, to be heterochromatic. HIGUCHI & al. (1981) found rRNA gene spacers dispersed throughout the human genome. Similar conditions would facilitate, if present in heterochromatin blocks of *Allium* species, transposition of rDNA to these sites. It might be that strain-specific increases of the frequency of transpositions of rDNA to non-satellite chromosomes (in top onion strains 124 and 437) is directly correlated to the loss of satellites in these strains.

Appendix

Site Specificity of Silver Staining as Compared to Giemsa Banding. Many authors believe that some Giemsa- or C-bands, and especially the so-called N-bands, occur in chromosome regions identical to those which become labelled by $AgNO_3$. Concerning the specificities of Giemsa banding and silver staining the following observations were made: In *Vicia faba*, for instance, the secondary constrictions of the satellite chromosome pair, harbouring the only active NORs of the genome (SCHUBERT & al. 1979), may appear covered by a Giemsa band with a high variability of appearance, depending on the pretreatment procedure (DÖBEL & al. 1978). After application of the trypsin-urea method, this region is the most variable of the so-called Giemsa marker bands. The visualization frequency of this band is not correlated with that of the other bands observed in the satellite chromosome. After application of the technique described by

SCHWARZACHER & al. (1980), this band is one of the most pronounced bands of the karyotype. After pretreatment with BaOH for detection of centromeric heterochromatin it is the only non-centromeric band observed in the karyotype (DÖBEL & al. 1978). Recently, we found a pretreatment procedure by which this region becomes exclusively stained by Giemsa ("N-banding", see Fig. 5). In stretched satellite chromosomes of *Vicia faba*, this Giemsa banded region seemed to be divided (a small part on the proximal and a larger on the distal border of the secondary constriction) or shifted entirely to the proximal part of the satellite, leaving the secondary constriction "open", i.e., unstained (see also SCHUBERT & RIEGER 1980).

Phenomena similar to those reported for *Vicia faba* also occur in *Allium* and, in this case, a clearcut interpretation is possible. In *A. fistulosum*, the secondary constriction is marked proximally by a small heterochromatic region and distally by the completely heterochromatic satellite. In *A. cepa*, the heterochromatic satellite is smaller and the proximal heterochromatic band larger (SCHUBERT & al., 1983), and in strongly condensed (short) chromosomes the secondary constriction also seems to be darkly stained, but this never occurs in stretched chromosomes.—Silver impregnation, however, is strictly confined to the nucleolus organizing region, which appears to be achromatic in Feulgen- or carmine-stained chromosomes. This becomes especially clear in *A. fistulosum* and *A. cepa* satellite chromosomes (Fig. 6).

Our interpretation that Giemsa banding and silver banding do not stain the same chromosomal regions (see Fig. 5) gains additional support from the observation that $AgNO_3$ stains both the secondary constriction itself and also nucleoli (indicating actively transcribing rDNA), whereas the various Giemsa techniques used in this investigation all failed to stain nucleoli darkly. PATHAK (1980) arrived at similar conclusions concerning the relation of silver stained NORs and C- or G bands in human and other mammalian chromosomes[1].

Concerning the occasional staining of centromeres by $AgNO_3$ we suggest that either NOR- and centromere staining have a different material base, or the non-histon proteins taken to be responsible for NOR staining are not proteins connected specifically with rRNA-synthesis (HOWELL 1977), but proteins causing silver staining by their quantity (high in NORs, less in centromeres, and insufficient for detection in the residual chromatin) rather than by their quality. The argument of HIZUME & al. (1980) that pectinase-cellulase maceration is essential for silver impregnation of plant NORs does not hold, since in our experiments roots were always hydrolyzed (1 n HCl, 60 °C, 11 min) prior to silver staining.

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[1] *Note added in proof.* For different amphibian species, however, SCHMID (Chromosoma **87**, 327—344, 1982) found that Giemsa C-banding may, in dependence of the species investigated, label either the heterochromatin adjacent to the NOR or the NOR plus adjacent heterochromatin.

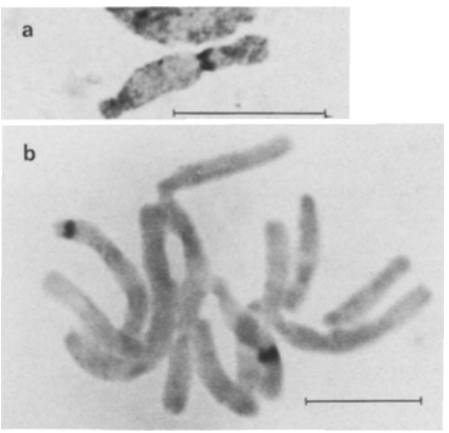

Fig. 5. N-banding in *Vicia faba*. *a* Single satellite chromosome of the reconstructed karyotype ACB (note the band extension including the proximal heterochromatic part of the satellite); *b* heterozygous metaphase with secondary constriction in two differently reconstructed satellite chromosomes (left: chromosome II of karyotype Q_{II}, right: chromosome III of karyotype ACB; this banding pattern was found during *in situ* hybridization experiments described in the Methods section, p. 292). Bars = 10 μm

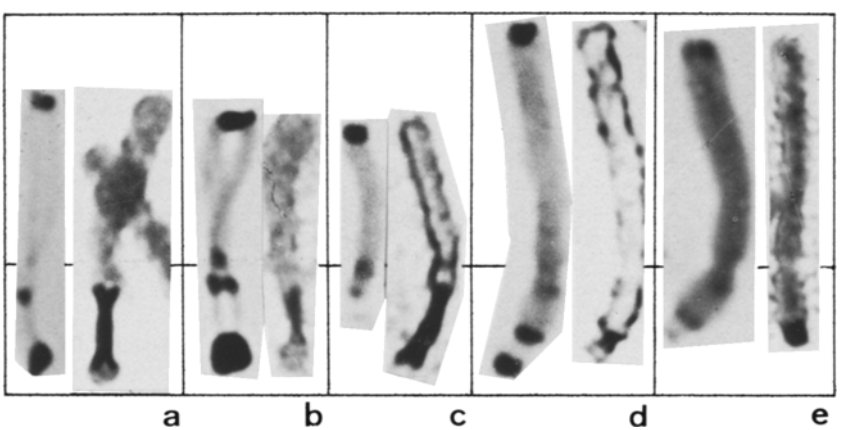

Fig. 6. Satellite chromosomes (magnification ×4000) after Giemsa banding (left) and after silver-staining of NOR (right). *a A. fistulosum; b* the *A. fistulosum* satellite chromosome of top onion strain 80; *c* top onion strain 437, the former *A. fistulosum* satellite chromosome without satellite; *d A. cepa; e* the former *A. cepa* satellite chromosome without satellite of top onion strain 124

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