Localization of Ribosomal Gene Sites in the Standard Nucleolar Chromosomes of *Allium cepa*\(^1\)

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**Key Words:** *Allium cepa.*—Feulgen staining, gene sites, in situ hybridization, nucleolar, ribosomal RNA, secondary constriction, silver banding.

**Abstract:** Nearly terminal sites of Feulgen achromasia coincide with nearly terminal sites of silver banding in the short arms of the subtelocentric pair of chromosomes of *A. cepa*. Autoradiographic silver grains after in situ rRNA/DNA hybridization cover a more extended area at this site, thereby giving the molecular basis for the staining results. From these staining results we conclude that the transcriptionally active rRNA genes of the standard nucleolar chromosomes are located in the secondary constriction.

The exact localization of ribosomal RNA (rRNA) gene sites in the nucleolar chromosomes of *A. cepa* is unknown till now as verified by MAGGINI et al. in 1978. In the cytological literature various karyotypes have been described thus giving evidence for a chromosomal polymorphism of the nucleolar organizer region concerning her number and morphological feature (BATTAGLIA 1957, NODA 1953, STACK 1974, VOSA 1976). We have used Feulgen staining, in situ rRNA/DNA hybridization and silver banding to clear up the chromosomal location of rRNA genes and their activity within nucleolar organizer regions in the standard nucleolar chromosomes of the commercial cultivar “Stuttgarter Riesen”. In another plant species (*Vicia sativa*) we found with these cytological techniques a coincidence of Feulgen achromatic secondary constrictions, radioactively labelled ribosomal gene sites and silver precipitates (BURGER & KNÄLMANN 1980).

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Material and Methods

Bulbs of *A. cepa* cv. 'Stuttgarter Riesen' were purchased in markets. Preparation of plant material, the technique of in situ hybridization and the staining procedures were previously described in detail (BURGER & KNÄLMANN 1980).

Results

After Feulgen staining only one pair of chromosomes carries achromatic secondary constrictions. They are located at the end of the short arm to which very small satellites are attached (Fig. 1). This satellite (SAT)-chromosome is the only subtelocentric chromosome in the genome, thus identical with chromosome M*7* as defined by BATTAGLIA (1957). In the metaphase plate shown as in all others observed homologous SAT-chromosomes are heteromorphic concerning the nucleolar organizer region. In most metaphase plates only one chromosome carries a distinct satellite which forms little granules. The inset of Fig. 1 shows such a SAT-chromosome in late metaphase. To both chromatids of the short arm granulated satellites are obviously attached with faintly stained threats. Such threats are called attachment fibres and are well known in *Allium cepa* as reported by TJIO & LEVAN (1956).

After 18/25S 3H rRNA/DNA in situ hybridization all metaphase plates under observation are labelled on chromosome M*7*. A scatter of silver grains round the end of the short arm of the nucleolar chromosome exhibits the site of the ribosomal genes (Fig. 2). The area, which is covered by silver grains, is of greater expansion than the region of the

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Fig. 1. Metaphase plate of *A. cepa* root tip (*2n* = 16) after Feulgen staining. The only pair of subteloentric chromosomes is constricted at the very end of the short arms (arrows) thus forming microsatellites. Both, achromatic secondary constriction and satellite seem to be heteromorphic. The inset shows the nucleolar chromosome of another metaphase plate with satellite granules and attachment fibres. Bars, 10 μm

Fig. 2. Subteloentric chromosomes of *A. cepa* with silver grain depositions after 3H 18/25S rRNA in situ hybridization (*~2 \times 10^6\ dpm/\mu g*). One subteloentric chromosome of the metaphase plate (right part) as well as that of the inset show distinctly that the silver grain depositions cover the nucleolar organizer region completely. The other subteloentric chromosome (left part of the metaphase, center) which is not so perfectly spreaded is labelled too. Bars, 10 μm

Fig. 3. Silver precipitates in the nucleolar constriction of the subteloentric chromosomes of *A. cepa* after Ag-banding. The pair of homologous chromosomes show satellites of different size adjacent to the silver precipitates (arrows). Bars, 10 μm