Observations of a specific rDNA locus behaviour during the cell cycle were made by fluorescent in situ hybridisation (FISH) in 12 Lupinus species. Due to the pattern of chromatin de-condensation in that locus, the number of relevant sites in interphase nuclei was twice as high as the number of signals on metaphase chromosomes. The description of successive phases and an attempt of an explanation are given.¹

Key words: cell cycle, fluorescent in situ hybridisation, Lupinus, 18S-25S rDNA.

The genus Lupinus (Fabaceae) comprises numerous species and botanical forms growing in the Old and New World, both wild forms and economically important crops. The phylogeny of the genus and relationships among contemporary species are unclear (GLADSTONES 1998). The identification of individual lupin chromosomes by conventional cytology is not possible due to their high numbers, gradient in size, and similar morphology (ATKINS et al. 1998). Hence, studies on physical mapping of Lupinus genomes have been undertaken. The presented results contribute to the work on the localisation of 18S-25S and 5S rDNA loci by the two-target fluorescent in situ hybridization (FISH) (NAGANOWSKA, ZIELIŃSKA 2002, NAGANOWSKA et al. 2003 and unpublished results). Within the framework of these studies, a large intense 18S-25S rDNA signal was observed in 12 species of the genus Lupinus (besides 5S rDNA loci and – in some

¹The results were presented at the 10th National Cytogenetic Conference (Poznań, June 5-6, 2003).
species – additional small signals of 18S-25S rDNA). The aim of the present work was to analyse that specific signal, with its pattern of de-condensation throughout the cell cycle similar in all the lupins studied.

Seeds of species belonging to two different Lupinus groups were used:
1) Scabrispermae (rough-seeded): L. atlanticus Gladst. (2n = 38), L. cosentinii Guss. (2n = 32), L. digitatus Forsk. (2n = 36), L. palaestinus Boiss. (2n = 42), L. pilosus Murr. (2n = 42), L. princei Harms (2n = 38);

The preparation of chromosome spreads and FISH procedure were based on protocols of Schwarzer et al. (1994), applied to Lupinus chromosomes (Naganowska, Zielinska 2002). The molecular probe 18S-25S rDNA from Arabidopsis thaliana was used, labelled by nick translation (Nick Translation System, Roche) with biotin or digoxigenin. The signals were revealed with anti-digoxigenin-FITC (Roche) or avidin-Rhodamine (Vector), respectively. Chromosomes were counterstained with DAPI. The preparations were examined with an OLYMPUS BX 60 Research System Microscope. The images were acquired with a black and white CCD camera, interfaced to a PC running the analysis SIS 3.0 software (Soft Imaging System).

We found that instead of two large and intense 18S-25S rDNA signals revealed on metaphase chromosomes, four relevant sites were often present in the interphase. Small rDNA signals observed in the interphase nucleus may be low-copy-number loci, invisible in the metaphase because of the more condensed chromatin structure (Snowdon et al. 1997). However, on the basis of the observations throughout the cell cycle, it turned out that this difference was due to the de-condensation of the secondary constriction within the large 18S-25S rDNA signal on a satellite chromosome pair that covered the satellite, nucleolar organising region (NOR) and a considerable part of the chromosome arm (Figures 1a-f). In the interphase, in place of one signal, two clearly distinct sites were observed, often located in the distant regions of the nucleus, outside the large nucleolus visible as a darker area. These were probably clusters of compact chromatin called perinucleolar knobs (Jordan 1984), comprising inactive rDNA regions of the satellite and the part of the chromosome arm adjacent to NOR. Thin filaments labelled by hybridisation were sometimes visible, joining two respective 18S-25S rDNA fragments (Figure 2). That should be the highly de-condensed chromatin of the transcriptionally active NOR, located inside the nucleolus. During successive phases the gradual re-condensation was observed into one large 18S-25S rDNA metaphase signal, composed of two subunits connected with the secondary constriction. The NOR on the relevant chromosome is supposed to be active as in most species studied it is the unique pair of the re-
revealed 18S-25S rDNA loci, and in the remaining species the additional signals are much smaller.

HAJDERA et al. (2003), while analysing 25S rDNA loci in *L. angustifolius* and *L. cosentinii* by silver staining method, stated that the locus in question is active. They found images of 25S rDNA de-condensation similar in *L. angustifolius*, but different for *L. cosentinii*.

De-condensation of the ribosomal gene chromatin during the cell cycle proceeds in different ways depending on cell type and species (LEITCH 2000). The pattern resembling lupins was observed, e.g., in *Petunia hybrida* (MONTIJN et al. 1998). However, in *Petunia* in the prophase the signals were small and in the interphase the smaller ones were hardly visible (often only one of them was present). In lupins two clear compact signals (corresponding to one large in the metaphase) were present during the whole prophase, as well as in the interphase nuclei. It is probably due to the fact that in such large 18S-25S rDNA regions there is a lot of inactive chromatin forming big clusters – the perinucleolar knobs.

Analyses of rDNA loci by FISH used to be made at different cell cycle stages, also in the interphase, and the number of the signals usually reflects their number on chromosomes (e.g., WEISS, MALUSZYNsKA 2000, in autotetraploid plants).

Figure 1a-f. 18S-25S rDNA signals in the successive phases of mitosis in *Lupinus pilosus*

- a = interphase, b-c = prophase, d-e = prometaphase, f = metaphase (probe – FITC; chromosomes – DAPI). Bar = 10 μm.
Our results indicate that the number and relative location of rDNA sites in interphase nuclei do not necessarily correspond to the relationships in the metaphase. In studies of other genera such a possibility should also be taken into consideration.

REFERENCES


