Mapping Phaseolin Genes to Polytene Chromosomes by Fluorescence *in situ* Hybridization (FISH)

Mario Nenno(1), Klaus Schumann, Walter Nagl

Division of Cell Biology, The University, P.O.B. 30 49, 6750 Kaiserslautern, Germany
(1) e-mail: nenno@rhrk.uni-kl.de, (2) after July 1st, 1993 use zipcode 67653

INTRODUCTION

The karyotype of *Phaseolus* (2n = 2x = 22) is still under investigations, not at least due to the small size of mitotic chromosomes and the low frequency of cells in mitotic divisions.

To avoid these difficulties an alternative lies in the study of the polytene chromosomes of the embryo suspensor *of Phaseolus coccineus* and *Phaseolus vulgaris* (Nagl, 1974). The major advantage is that they are polyploid due to endoreduplication cycles, which lead to lateral amplification of DNA sequences. Furthermore, polytene chromosome become 30 times longer than the metaphase chromosomes. Although their length and distribution of heterochromatin can be used for the establishment of a preliminary karyotype, these characteristics show some variability and do not allow the identification of individual chromosomes in any case.

In a new approach we try to establish a karyotype of *Phaseolus* not based on morphological features but on molecular markers, and therefore called molecular karyotype. Molecular markers in this sense are genes or other DNA sequences which are mapped to chromosomes. To localize these markers they were labeled, hybridized to the chromosomal DNA and detected directly on the chromosome. When fluorescence dyes are used for detection this thechnique is called fluorescence *in situ* hybridization (FISH). A disadvantadge of FISH was so far, that it could be well used for detection of repetitive genes and DNA sequences, but not for low copy and single copy genes.

In this work we report for the first time the mapping of phaseolin genes (low copy genes) to polytene chromosomes of *Phaseolus coccineus* by fluorescence *in situ* hybridization.

MATERIALS AND METHODS

The embryo suspensors were taken from seeds of unripe pods of *Phaseolus coccineus* cv. Hammond's Dwarf Scarlet. Preparation of polytene chromosomes was made from the big basal suspensor cells. Prior to *in situ* hybridization chromosome preparations were digested with pepsin/HCl to degrade cytoplasmatic and chromosomal proteins.

The clone pTA250 carrying a 8.8kb repeat unit of ribosomal RNA genes (rDNA) from *Triticum aestivum* var. Chinese Spring (Gerlach and Bedbrook, 1979) was used for test hybridization to monitor the effects of protein degradation.

The DNA probe for mapping phaseolin genes was the 3kb insert of the clone AG-pPVPh 3.0, which includes a 1.9kb coding sequence for ß-phaseolin from *Phaseolus vulgaris* cv. Tendergreen (Slightom et al., 1983).

Probes were labeled with biotin and detected with avidin-FITC (fluorescein isothiocyanat) after *in situ* hybridization. Signals were amplified with biotinylated antibodies and avidin-FITC.

RESULTS AND DISCUSSION

Chromosome preparations were digested with pepsin/HCl to degrade proteins, in order to increase accessibility for *in situ* hybridization and detection. After this pretreatment the test system of the rDNA probe showed increased density and number of signals spots. When the same pretreatment was used with the phaseolin probe, we got hybridization signals on two homologous chromosomes. According to their size and the distribution of heterochromatin, they were classified as chromosome no. 7. In a previous report we identified them as no. 3, but the new data support a classification as chromosome no. 7.

This is the first time that low copy genes like those for phaseolin have been localized on polytene chromosomes of plants by fluorescence *in situ* hybridization (FISH). In general, radioactive *in situ* hybridization is used for mapping low and single copy genes, but FISH has several advantages, like higher spatial resolution, short detection time, and the possibility to detect serveral probes on one slide.

Now we are going to map further low copy genes. In combination with the detection of different probes with different fluorochromes, this will allow us to establish a molecular karyotype.

This technique would also be of interest for mapping RFLP markers to chromosomes, and to reduce the current number of fifteen linkage groups (Nodari et al., 1993) to the eleven pairs of chromosomes. So FISH can combine the genetic and the physical map of *Phaseolus*.

ACKNOWLEDGEMENTS

We thank Dr. Jerry L. Slightom (Madison, WI) for providing the phaseolin clone AG-pPVPh 3.0. This work was supported by the Deutsche Forschungsgemeinschaft (DFG, grant no. Na 107/9-2).

REFERENCES

Nagl, W. (1974). The *Phaseolus* suspensor and its polytene chromosomes. Z. Pflanzenphysiol. 73: 1-44.

Nodari, R.O., Tsai, S.M., Gilbertson, R.L., Gepts, P. (1993). Towards an integrated linkage map of common bean 2. Development of an RFLP-based linkage map. Theor. Appl. Genet. 85: 513-520.

Schumann, K., Baumann, A., Nagl, W. (1990). Localization of phaseolin genes in the polytene chromosomes of *Phaseolus coccineus* (*Leguminosae*). Genetica 83: 73-76.

Slightom, J.L., Sun, S.M., Hall, T.C. (1983). Complete nucleotide sequence of a French bean storage protein gene: Phaseolin. Proc. Natl. Acad. Sci. USA 80: 1897-1901.

Request

Genes and DNA sequences for fluorescence *in situ* hybridization (FISH)

I am intrested in establishing a molecular karyotype of *Phaseolus* by molecular markers.
The ideal molecular markers would be DNA sequences with a length of several kilo base pairs cloned in the plasmid vector pUC19. Sequences with a high copy number in the genome are of special interest.

But also all others would be gratefully accepted.

If you like to provide me with sequences, please contact:

Mario Nenno
University of Kaiserslautern
Division of Cell Biology
P.O.B. 30 49
6750 Kaiserslautern (1)
Germany
e-mail: nenno@rhrk.uni-kl.de
(1) after July 1993 use zipcode 67653