ORGANISATION AND COMPOSITION OF THE PLANT NUCLEAR MATRIX. CHARACTERISATION AND SUBCELLULAR DISTRIBUTION OF A MAR-BINDING PROTEIN: AcMFP1.

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Nucleic acid metabolism is spatially ordered in the nucleus by association to the nuclear matrix, a scaffolding structure formed by the core filaments of the nucleoskeleton to which multimeric complexes attach [1]. While the importance of the nuclear matrix in nuclear function is increasingly recognised [1; see 2, 3 for plants], little is known about nuclear matrix organisation, and even less about its proteins involved in the organisation of nuclear domains in plants [4]. Resinless sections have revealed an anastomosed network of periodic filaments with dense aggregates of variable sizes forming the plant nucleoskeleton [5]. Immunolabelling reveals intermediate filaments (IF) as components of this network [4, 5]. Homologous of mammalian A and B type lamins [6, 7], and of NuMA were identified in the onion nucleoskeleton [5]. Although some proteins known to have MAR-binding activity have been immunologically detected in plant nuclear matrices, like lamin B itself, topoisomerase II [4] and nucleolin [8], only a few genes for plant MAR-binding proteins have been cloned. Between them MFP1 (MAR-binding Filament-like Protein 1) [9] and two MAR binding proteins of the pea nuclear matrix, probably related to rDNA [10]. MFP1 is conserved amongst plants [11] and shares a high degree of similarity with filament-like proteins from animal and yeast. The secondary structure analysis indicates that all MFP1 proteins contain an extended amphipathic α-helical domain that has been proposed to fold into a coiled-coil protein structure, a DNA-binding domain at the carboxy terminus, and an N-terminal transmembrane domain. MFP1 reveals 13 motifs of the consensus recognition motif of casein kinase II; a kinase associated to the nuclear matrix, and is an “in vitro” substrate of this enzyme [9]. Although the interspecific conservation of the sequences is only about 40%, the domains involved in membrane attachment and DNA-binding are highly conserved, implying that they have potential important roles in cells, not only in the membrane system, but also in chromatin organisation [11]. By using domain-specific antibodies, we analysed the MFP1-like proteins in nuclei and nuclear matrices from onion proliferating cells (Allium cepa). Two AcMFP1 proteins with different molecular masses and solubilities were detected. The most abundant is a 90 kD basic protein, presenting several levels of phosphorylation in 2D-blots. In spite of their sequence similarity, AcMFP1 differs from onion lamins and AcNuMA in its Mr and pl values, number of isoforms and subcellular distribution. [4, 5,
MFP1 is highly insoluble, and localises in the nucleus and nuclear matrix with a speckle pattern, similar to that of replication factories as detected by PCNA and BrdU labelling in confocal microscopy (Figs. a-i) [12]. High-resolution immunolocalization of MFP1 by electron microscopy identifies the speckles as a new category of nuclear bodies, ultrastructurally similar to the replication factories of animal cells (Fig. k, l) [13], and also reveals the association of AcMFP1 to the core filaments of the nucleoskeleton in resinless sections (Fig. j). Our data provide the first evidence of the presence of a MAR-binding protein in the replication factories of plant nuclei and nuclear matrices.

Figures. Topological distribution of AcMFP1 in the nuclei (a through g) and nuclear matrices (h) from cycling cells, by immunolabelling with a-MFP1 serum. Confocal sections of two proliferating nuclei in late replicating phase (S3), displaying a pattern of MFP1 distribution in large speckles, probably corresponding to replicon clusters, similar to those shown by PCNA labelling in the same phase. g: projection of the six sections shown. Bar=5 mm. h and i: projections of confocal images of nuclear matrices. h: MFP1 labelling with a S3 pattern. i: PCNA labelling of a nuclear matrix on a similar stage. Bars=1 mm. j: Resinless-section displaying a pattern of MFP1 labelling in association with the filaments of the nuclear matrix, similar to the non-clustered replication factories. k and l: Conventional sections displaying MFP1-bodies/replication factories after either MFP1 (k)/PCNA (l) labelling. Bars=0.1mm.

REFERENCES