

Fine structural analyses of pancreatic acinar cell nuclei from mice fed on genetically modified soybean

M. Malatesta,* M. Biggiogera,^o E. Manuali,[#] M.B.L. Rocchi,[§] B. Baldelli,* G. Gazzanelli*

*Istituto di Istologia e Analisi di Laboratorio, and [§]Istituto di Biomatematica, University of Urbino "Carlo Bo", Urbino; ^oLaboratorio di Biologia Cellulare, Dipartimento di Biologia Animale, University of Pavia, and Istituto di Genetica Molecolare del CNR, Pavia; [#]Istituto Zooprofilattico Sperimentale dell'Umbria e delle Marche, Perugia; Italy



©2003, European Journal of Histochemistry

We carried out ultrastructural morphometrical and immunocytochemical analyses on pancreatic acinar cell nuclei from mice fed on genetically modified (GM) soybean, in order to investigate possible structural and molecular modifications of nucleoplasmic and nucleolar constituents. We found a significant lowering of nucleoplasmic and nucleolar splicing factors as well as a perichromatin granule accumulation in GM-fed mice, suggestive of reduced post-transcriptional hnRNA processing and/or nuclear export. This is in accordance to already described zymogen synthesis and processing modifications in the same animals.

Key words: cell nucleus, exocrine pancreas, genetically modified soybean, splicing factors

Correspondence: Dr. Manuela Malatesta, Istituto di Istologia e Analisi di Laboratorio, University of Urbino, via Zeppi s.n., 61029 Urbino, Italy. Phone: international +39.0722.320168. Fax: international +39.0722.322370. E-mail: malatesta@uniurb.it

Paper accepted on July 30, 2003

European Journal of Histochemistry
2003; vol. 47 issue 4 [Oct-Dec]: 385-388

Genetically modified (GM) crops, in which new genes have been inserted into the original genome, are nowadays utilized all over the world both for animal and human consumption. No direct evidence that GM food may represent a possible danger for health has been reported so far; however, the scientific literature in this field is quite poor and heterogeneous. Nutritional evaluations of GM crops have been carried out and the potential problems related to the introduction of GM food in animal and human diets, such as antibiotic resistance and allergenicity, have been discussed (Halford & Shewry, 2000). In addition, some authors have investigated the fate of ingested foreign DNA (e.g. Schubbert et al., 1994); others have studied the optimal conditions to ensure sufficient DNA fragmentation to a size where it would be unlikely to be stably transferred to host cells (Chiter et al., 2000). On the other hand, reports on a possible effect of GM diet on the morphological patterns of cells and tissues are few.

In previous studies we described significant modifications of some nuclear features in hepatocyte nuclei of mice fed on GM soybean (Malatesta et al., 2002a). Moreover, we reported that a diet containing significant amounts of GM food seems to influence the zymogen synthesis and processing in mouse pancreatic acinar cells (Malatesta et al., 2002b). In this view, it seemed interesting to evaluate also some nuclear parameters which could be influenced by the diet. In the present study, we have hence carried out ultrastructural morphometrical and immunocytochemical analyses on pancreatic acinar cell nuclei from the same mice, in order to investigate possible structural and molecular modifications of nucleoplasmic and nucleolar constituents which may be related to the already described cytoplasmic changes.

Materials and Methods

Pregnant Swiss mice were fed on a standard laboratory chow containing 14% GM soybean obtained

by the insertion of the bacterial CP4 EPSPS (5-enolpyruvylshikimate-3-phosphate synthase, an enzyme obtained from *Agrobacterium* sp. strain CP4) gene conferring a high level of tolerance to glyphosate, the active ingredient of the herbicide Roundup (Padgett et al., 1995). In parallel, other pregnant mice were fed on the same diet with wild soybean. The respective litters (24 female mice) were fed on the parental diet (12 controls and 12 GM-fed) and sacrificed when 1, 2, 5 or 8 months old. Fragments of pancreas were aldehyde-fixed and embedded in LRWhite resin as previously described (Malatesta et al., 2002b). Ultrathin sections were stained with the EDTA method (Bernhard, 1969) to visualize RNP constituents and observed in a Philips CM 10 electron microscope.

Morphometrical analyses were performed by using a computerised image analysis system (Image Pro-Plus for Windows 95) on 240 electron micrographs (10 from each animal) (x20,000) of acinar cell nuclei containing at least one nucleolus. Nuclear and nucleolar areas, percentages of fibrillar centres (FCs), dense fibrillar component (DFC) and granular component (GC) per nucleolus, FC area, index of nuclear shape irregularity (the ratio between the measured perimeter and the circumference of the equivalent circle), perichromatin granule density (PG/ μm^2), nuclear pore density (NP/ μm) were considered (Malatesta et al., 2002a). For each variable a two-way ANOVA test (with age and food factors) was performed. The ANOVA models included an interaction term between the factors. Significance level was fixed at $\alpha=0.05$.

The fine intranuclear distribution of some transcription and splicing factors was investigated by

using specific antibodies on 5 month-old animals, as a sample. Mouse monoclonal antibodies directed against the polymerase II (Research Diagnostics Inc.), the (Sm)snRNP (small nuclear ribonucleoprotein) core protein (Lerner et al., 1981), the non-snRNP splicing factor SC-35 (Sigma-Aldrich) and the nucleolar protein fibrillar (Cytoskeleton Inc.) were revealed by gold-conjugated antibodies (Jackson ImmunoResearch Laboratories Inc.) as described in Malatesta et al. (2002a). All samples were stained with the EDTA technique (Bernhard, 1969). The labelling density (gold grains/ μm^2) over cytoplasm, nucleoplasm, nucleolus and, for anti-fibrillar antibody, DFC was evaluated on 15 micrographs (x26,000) from each animal. For background evaluation, the resin outside the tissue was considered. Statistical comparisons were performed by the Kruskal-Wallis one-way ANOVA test ($p \leq 0.05$).

Results

Pancreatic acinar cell nuclei from GM-fed and control mice showed similar features. Cell nuclei generally contained large clumps of condensed chromatin (Figure 1). In the nucleoplasm, perichromatin fibrils (PFs) and perichromatin granules (PGs) were distributed along the heterochromatin borders, while interchromatin granule (IG) clusters occurred in the interchromatin space. The nucleoli generally displayed roundish shapes and compact arrangement, with some FCs surrounded by DFC and abundant GC. The morphometric data (Table 1) revealed that many variables changed significantly in relation to the food and/or to the age-food term. In detail, the nuclear and nucleolar area as well as the percent-

Table 1. Means \pm SE values of variables considered in pancreatic acinar cells. Areas are expressed as μm^2 . Different symbols indicate statistical significance for complete model (*), age ($^{\circ}$), food ($^{\wedge}$) and interaction term age-food (#). C: control animals.

Age	Nucleus area (* $^{\circ}$)	Shape index (* $^{\circ}$ #)	PG density (* $^{\circ}$ $^{\wedge}$ #)	Nucleolar area (* $^{\circ}$)	FC area (* $^{\circ}$ $^{\wedge}$ #)	FC % (* $^{\wedge}$)	DFC % (* $^{\circ}$)	GC % (* $^{\circ}$)	Pore density (* $^{\circ}$ #)
1 month (C)	15.33 \pm 0.95	1.26 \pm 0.04	5.90 \pm 0.50	1.08 \pm 0.12	0.026 \pm 0.004	0.97 \pm 0.46	24.35 \pm 1.72	74.67 \pm 1.73	0.56 \pm 0.07
1 month	14.32 \pm 0.54	1.23 \pm 0.04	4.52 \pm 0.49	1.18 \pm 0.13	0.026 \pm 0.003	1.99 \pm 0.49	21.89 \pm 1.86	76.12 \pm 1.88	0.66 \pm 0.06
2 months (C)	15.84 \pm 0.93	1.31 \pm 0.03	3.66 \pm 0.45	1.26 \pm 0.13	0.021 \pm 0.002	1.78 \pm 0.48	22.16 \pm 1.82	76.07 \pm 1.84	0.91 \pm 0.07
2 months	15.69 \pm 0.96	1.23 \pm 0.04	8.25 \pm 0.65	0.98 \pm 0.11	0.008 \pm 0.002	2.89 \pm 0.41	25.95 \pm 1.53	71.16 \pm 1.54	0.56 \pm 0.07
5 months (C)	16.51 \pm 0.95	1.26 \pm 0.04	5.49 \pm 0.50	1.23 \pm 0.12	0.021 \pm 0.003	1.01 \pm 0.46	24.77 \pm 1.72	74.23 \pm 1.73	1.25 \pm 0.07
5 months	16.33 \pm 0.97	1.16 \pm 0.04	10.69 \pm 0.51	1.11 \pm 0.14	0.008 \pm 0.002	2.34 \pm 0.52	21.68 \pm 1.95	75.97 \pm 1.96	0.86 \pm 0.07
8 months (C)	18.29 \pm 0.89	1.19 \pm 0.03	7.10 \pm 0.46	1.38 \pm 0.11	0.027 \pm 0.003	1.74 \pm 0.43	19.05 \pm 1.63	79.21 \pm 1.64	1.22 \pm 0.06
8 months	19.51 \pm 0.98	1.14 \pm 0.04	13.22 \pm 0.51	1.45 \pm 0.14	0.007 \pm 0.003	1.85 \pm 0.53	18.38 \pm 1.99	79.76 \pm 2.01	0.83 \pm 0.07

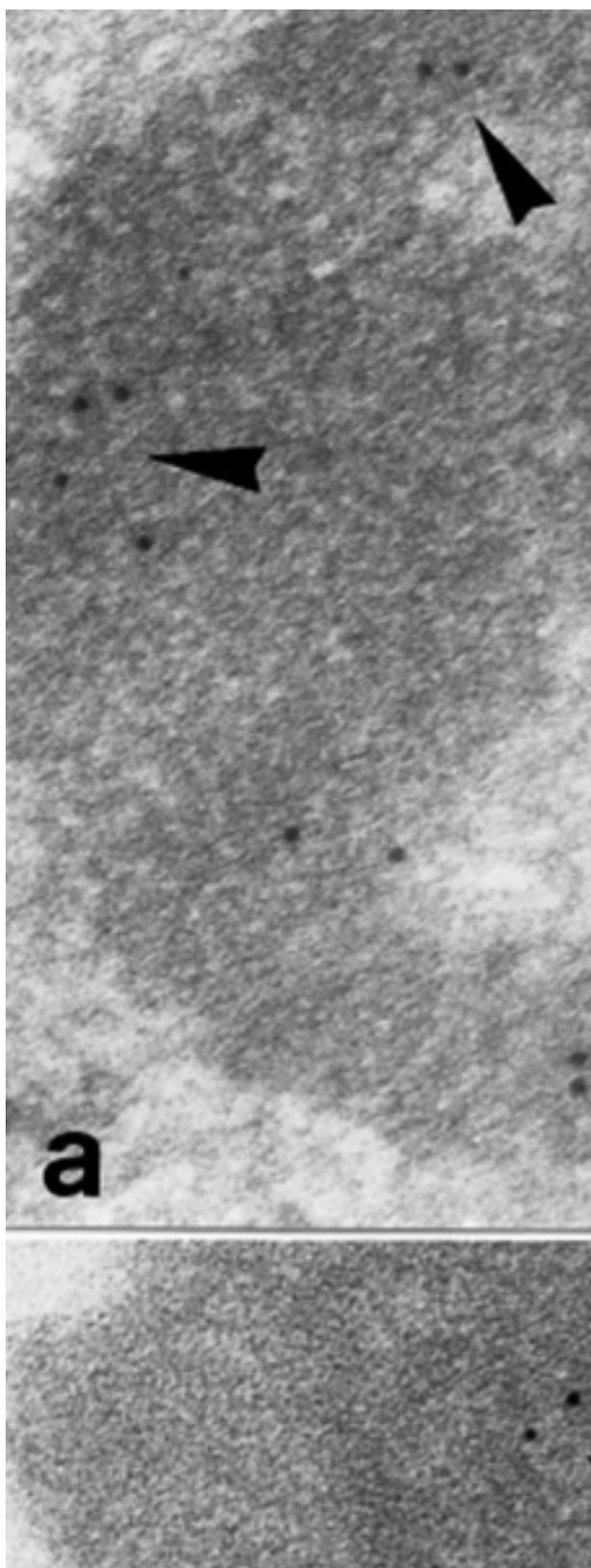


Figure 1. Pancreatic acinar cell nuclei. The anti-fibrillarin immunolabelling (arrowheads) is clearly stronger in nucleoli of control (a) than GM-fed (b) mice. Note the numerous perichromatin granules (arrows) in b. Bars 0.5 μ m.

Table 2. Mean values \pm SE of labelling densities (gold grains/ μ m²) obtained with anti-polymerase II, anti-(Sm)snRNP, anti-SC-35 and anti-fibrillarin antibodies on pancreatic acinar cell nuclei from control and GM-fed 5 month-old mice. In each column, values identified by common symbols (*, #, §) are not significantly different from one another. C: control animals.

	Cytoplasm	Nucleoplasm	Nucleolus	DFC
Polymerase II	4.42 \pm 0.72* (C)	5.29 \pm 0.36* (C)	1.89 \pm 0.21* (C)	–
	3.64 \pm 0.47*	5.10 \pm 0.45*	2.30 \pm 0.30*	–
(Sm)snRNP	0.94 \pm 0.13 (C)	3.35 \pm 0.26 (C)	0.61 \pm 0.16 [#] (C)	–
	0.22 \pm 0.06	1.90 \pm 0.26	0.10 \pm 0.08 [#]	–
SC-35	4.28 \pm 0.85 (C)	6.83 \pm 1.18 (C)	1.94 \pm 0.23 [§] (C)	–
	2.07 \pm 0.94	3.28 \pm 1.16	1.54 \pm 0.37 [§]	–
Fibrillarin	3.60 \pm 0.49 [#] (C)	1.48 \pm 0.16 [#] (C)	4.13 \pm 0.30 (C)	14.19 \pm 5.33 (C)
	3.06 \pm 0.22 [#]	1.16 \pm 0.51 [#]	1.87 \pm 0.30	2.55 \pm 1.60

ages of DFC and GC changed in relation to the age only. The shape index and pore density changed in relation to the age-food interaction term also, the former decreasing and the latter increasing in GM-fed mice. The PG density increased and the FC area decreased in GM-fed mice in relation to all three parameters considered. Finally, the FC percentage increased in GM-fed mice in relation to the food only.

Immunocytochemical analysis demonstrated that no difference in polymerase II, snRNP, SC-35 and fibrillarin distribution occurred between GM-fed and control mice. As expected, polymerase II was exclusively associated to PFs; snRNPs were mainly associated to PFs and, to lesser extent, to IGs, moreover, a few snRNPs were found in nucleoli; SC-35 was specifically associated to PFs and IGs; fibrillarin accumulated in nucleolar DFC. Quantitative evaluation of immunolabelling (Table 2) revealed similar values in GM-fed and control mice for anti-polymerase labelling in the three cell compartments considered. Conversely, GM-fed mice showed significantly lower labellings for the nucleoplasmic splicing factors investigated in both cytoplasm and nucleoplasm, whereas the nucleolar values were similar. Finally, in GM-fed mice the labelling for fibrillarin decreased sharply in the whole nucleolus as well as in the DFC, while in the cytoplasm and nucleoplasm the signals remained unchanged.

Discussion

Our observations carried out on pancreatic acinar cell nuclei from control and GM soybean-fed mice demonstrate significant modifications of some

nuclear features in GM-fed mice.

As previously reported, the modifications of nuclear size are mostly related to age, probably because of the postnatal development of pancreatic acinar cells (Malatesta et al., 2002b). Similarly, the nucleolar size changes with age; it must be noted here that nucleolar size may be also influenced by circadian rhythms (Pebusque and Sèite, 1981): the mice here studied were sacrificed always at the same time of the day. On the other hand, FC area as well as the nucleolar surface occupied by FCs change in relation to food, in particular FCs increase in number but decrease in size in GM-fed mice. Such FC modifications generally occur when the nucleolar metabolic rate increases, however, this phenomenon is generally associated to a DFC increase (e.g. Schwarzacher and Wachtler, 1993). This is not the case of the mice here studied, where DFC and GC amounts do not change in relation to food. Moreover, fibrillarin, a component of the U3 snRNP complex, involved in several steps of rRNA processing (Kass et al., 1990), significantly decreases in nucleoli of GM-fed mice. Therefore, taken together, our data suggest a decrease in nucleolar activity in mice fed on GM soybean. Similarly, the nucleoplasmic splicing factors investigated — snRNPs, involved in early pre-mRNA splicing (Luhmann et al., 1990) and SC-35, required for spliceosome assembly (Fu and Maniatis, 1990) — are less abundant in GM-fed mice than in controls. On the other hand, no modification was found for polymerase II, responsible for mRNA transcription. Therefore, it could be hypothesised that in GM-fed mice a slowdown of post-transcriptional hnRNA processing would occur. This would be in accordance with the accumulation of PG — considered to constitute storage and/or transport sites of spliced mRNA (Vazquez-Nin et al., 1979) — in the nucleoplasm of these animals. Moreover, the lower shape index and pore density of GM-fed mice would imply a lower molecular trafficking between nucleus and cytoplasm. Consequently, a reduced nuclear export would further increase the number of PG even if splicing (i.e. a step upstream the complete PG formation) is partially hindered. The modifications observed in pancreatic acinar cell nuclei of

GM-fed mice could be related to the reduction in digestive enzyme synthesis and secretion previously described in the same animals (Malatesta et al., 2002b). Therefore, this study further supports the idea that a diet containing significant amounts of GM soybean can influence the pancreatic metabolism in mouse. Unfortunately, the reasons for such modifications remain so far unclear.

Acknowledgements

We express our gratitude to Dr. T.E. Martin for providing us with the anti-(Sm)snRNP antibody.

References

- Bernhard W. A new staining procedure for electron microscopical cytology. *J Ultrastruct Res* 1969; 27:250-5.
- Chiter A, Forbes JM, Blair GE. DNA stability in plant tissues: implications for the possible transfer of genes from genetically modified food. *FEBS Lett* 2000; 481:164-8.
- Fu XD, Maniatis T. Factor required for mammalian spliceosome assembly is localized to discrete regions in the nucleus. *Nature* 1990; 343: 437-41.
- Halford NG, Shewry PR. Genetically modified crops: methodology benefits regulation and public concerns. *Br Med Bull* 2000; 56:62-73.
- Kass S, Tyc K, Steitz JA, Sollner-Webb B. The U3 small nucleolar ribonucleoprotein functions in the first step of preribosomal RNA processing. *Cell* 1990; 60:897-908.
- Lerner EA, Lerner MR, Janeway CA, Steitz J. Monoclonal antibodies to nucleic acid-containing cellular constituents: probes for molecular biology and autoimmune diseases. *Proc Natl Acad Sci USA* 1981; 78:2737-41.
- Luhmann R, Kastner B, Bach M. Structure of spliceosomal snRNPs and their role in pre-mRNA splicing. *Biochim Biophys Acta Gene Struct Expression* 1990; 1087:265-92.
- Malatesta M, Caporaloni C, Gavaudan S, Rocchi MBL, Tiberi C, Gazzanelli G. Ultrastructural morphometrical and immunocytochemical analyses of hepatocyte nuclei from mice fed on genetically modified soybean. *Cell Struct Funct* 2002a; 27:173-80.
- Malatesta M, Caporaloni C, Rossi L, Battistelli S, Rocchi MBL, Tonucci F, et al. Ultrastructural analysis of pancreatic acinar cells from mice fed on genetically modified soybean. *J Anat* 2002b; 201:409-46.
- Padgett SR, Kolacz KH, Delannay X, Re DB, LaVallee BJ, Tinius CN, et al. Development identification and characterization of a glyphosate-tolerant soybean line. *Crop Sci* 1995; 35:1451-61.
- Pebusque MJ, Sèite R. Electron microscopic studies of silver-stained proteins in nucleolar organizer regions: location in nucleoli of rat sympathetic neurons during light and dark periods. *J Cell Sci* 1981; 51:85-94.
- Schubert R, Lettmann C, Doerfler W. Ingested foreign (phage M13) DNA survives transiently in the gastrointestinal tract and enters the bloodstream of mice. *Mol Gen Genet* 1994; 242:495-504.
- Schwarzacher HG, Wachtler F. The nucleolus. *Anat Embryol* 1993; 188: 515-36.
- Vazquez-Nin GH, Echeverria OM, Pedron J. Effects of estradiol on the ribonucleoprotein constituent of the nucleus of endometrial epithelial cells. *Biol Cell* 1979; 35:221-8.