Division and endopolyploidisation in intestinal nuclei during postnatal ontogenesis of *Caenorhabditis elegans* (Nematoda)

Yves CHARNAY * et † Jean-Louis BRUN

Laboratoire de Génétique Physiologique et Nématologie, Département de Biologie Générale et Appliquée, Laboratoire Associé au CNRS n° 92, Université Claude Bernard Lyon-I, 43, boulevard du 11 Novembre 1918, 69622 Villeurbanne Cedex, France.

SUMMARY

The intestinal nuclei of the free-living nematode, *Caenorhabditis elegans*, were examined for their DNA-content using Feulgen microdensitometry technique. An endopolyploid process was characterized and modalities were studied with regard to the postnatal ontogenesis. The process operates in apparent synchrony and each intestinal nucleus reaches a final ploidy level of 32 C (C : copy of haploid genome equivalent) in adult worm. Successive ploidy levels appear in correlation with the four stages of ecdysis occurring during nematode life. Functional significance of endopolyploidy in biological systems remains unclear. However, these results show endopolyploidization as a well defined process during ontogenesis.

RÉSUMÉ

Division et endopolyploïdisation des noyaux intestinaux au cours de l'ontogenèse postnatale du nématode Caenorhabditis elegans

Le contenu en DNA des noyaux intestinaux du nématode libre *Caenorhabditis elegans* est estimé par Feulgen microdensitométrie. Un processus endopolyploïde est caractérisé et les modalités en sont étudiées au regard de l'ontogenèse post-natale. Le processus s'opère en synchronie apparente et chaque noyau intestinal atteint un degré de ploïdie final de 32 C (C : copie de génome haploïde équivalent) chez le ver adulte. Les degrés de ploïdie successifs apparaissent en corrélation avec les quatre ecdysis effectuées au cours de la vie du nématode. La signification fonctionnelle de l'endopolyploïdie dans les systèmes biologiques n'est pas claire. Cependant, ces résultats montrent l'endopolyploïdisation comme un processus bien défini au cours de l'ontogenèse.

Endopolyploidy is a wide-spreading phenomenon in animal and plant kingdoms (Brodsky, 1977 ; Nagl, 1978). It results from a disturbance or complete omission of the mitotic process during the cell-cycle. The genome is partially or completely replicated in non dividing nuclei. In most cases, the number of successive replications appears well defined for one cell-type in a given species : this leads to a characteristic nuclear DNA content (Anissimov, 1976 ; Thomas & Brown, 1976).

A better knowledge of the modalities and functional consequences of endopolyploidization seems to be necessary in order to study the regulation of gene activity. Cytophotometry is an elegant method for quantitative studies on endopolyploid cell *in situ* (Leuchtenberger, 1962 ; Nagl, 1978) for which recently developed microdensitometers perform well (Altman, 1975 ; Brugal & Chassery, 1977). We characterized the endopolyploid process in intestinal nuclei of the free-living nematode *Caenorhabditis elegans*, by the Feulgen microdensitometry technique.

Materials and Methods

We have used a hermaphrodite stock of *C. elegans*, Bergerac strain (Nigon, 1945). This

* Present address : Laboratoire d'Histologie et d'Embryologie, Faculté de Médecine Lyon-Sud-Ouest, chemin du Petit Revoyet, 69600 Oullins, France.

bacteria-feeding species is routinely maintained on solid agar medium A1 (Brun, 1966) and in deep cultures in liquid Kitamoto medium (Kitamoto *et al.*, 1974). An axenic subpopulation was initiated with axenic eggs obtained after sodium hydroxide treatment of xenic adults, as described by Patel and Mac Fadden (1978). Stocks are maintained at 20° in sterile conditions according to Vanfleteren (1978). Morpho-anatomic criteria can be used to estimate the larval and adult stages of a given specimen (Kimble & Hirsh 1979 ; Singh & Sulston 1978 ; Sulston & Horvitz 1978).

In vivo observations

Living worms were observed under Nachet N.S. 400 microscope equipped with Nomarski differential interference contrast optics. The nematode is mounted in 50 µl of axenic media (with 15% Kollidon 90) previously spread on a microscope slide coated with agar. The cover slide is sealed with immersion oil. This system was described by Sulston and Horwitz (1978) and is sufficient to observe intestinal nuclei behaviour for several hours in minimum illumination through heat filter.

Cytology

We used "frottis technique" (Nigon 1945) with anterior and posterior dissections of the worm. The digestive tract was fixed for two minutes in Carnoy-2 fluid, and stained by Feulgen reaction (Dutt, 1976). For maximum standardization, all operations were performed in a climatized-room at 20° and ten slides were treated simultaneously. Optimal DNA-hydrolysis time with 6N HCl at 20° has been determinated from a previous Feulgen microdensitometer analysis. After one hour in Schiff reagent, slides were mounted in synthetic mounting medium (Eukitt, Sté Pontet Girard, 92115 Clichy). For estimating absorption back ground, non hydrolyzed specimens have been processed in the same conditions. *C. elegans* spermatozoa and human lymphocytes have been used as references.

Microdensitometric analysis

This analysis was made with a Quantimet 720 D system equipped with a microdensitometer module (Altman, 1975 ; Fischer & Bonn, 1972). It is fitted with Plumbicon camera on a Zeiss microscope Obj. 100, Eyepiece 10, Aperture 1.25 in Koller illumination at 543 mm. In these conditions, the densitometer determine extinction of picture points with 0.1 µm, of effective diameter.

Measurements are combined to give the total integrated extinction at the selected field. A special device (light pen) permits precise definition on a screen of the region to be investigated. Before measurement, a shading corrector ensures even illumination over the entire field. Microdensitometer calibration was done for each slide examined. DNA content (Arbitrary Unit = A.U.) corresponds to the extinction value given by the cytophotometer (Anissimov, 1976 ; Nagl, 1978 ; Sin & Pasternack, 1970). Stœchiometry and specificity of Schiff-reagent DNA chromophores in Feulgen reaction are generally accepted by most authors (Dutt, 1976 ; Gabe, 1968 ; Gauthier, 1976). Therefore the Beer and Lambert law is applicable.

Results

In vivo observations

During post embryonic development, *C. elegans* sheds its cuticle four times between the four larval stages L1, L2, L3, L4 and the adult stage (Singh & Sulston, 1978).

At hatching, the young worm L1 contains 20 intestinal nuclei. In the lateral view, the nuclei appear as two staggered files except the four anterior ones (Fig. 1). Other larval stages and the adult present the same spatial arrangement but with 34 (sometimes 32) intestine nuclei.

Post-embryological divisions of intestinal nuclei occur only between L1 and L2 larval stages according to a precise pattern described in *C. elegans* Bristol Strain (Sulston & Horvitz, 1978).

Table 1

C. elegans intestinal nuclei DNA content (A.U.) during postnatal ontogenesis

$\left(\text{Standard deviation} \pm 2\sqrt{\frac{s^2}{n}}\right)$,

$\bar{l}$ = body length mean in µm, n = nuclei examined, c = copy of haploid genome equivalent.

Stage	*l*	*n*	*DNA content (A.U.)*	*c*	*Number of intestinal nuclei per individual*
L1	300	30	116 ± 14	2	20
L2	500	32	229 ± 20	4	34
L3	600	72	481 ± 20	8	34
L4	700	58	1003 ± 31	16	34
Adult	1100-1300	330	2071 ± 33	32	34

Table 2

C. elegans germ-cells DNA content (A.U.), * Delavault (1959), n = nuclei examined, c = copy of haploid genome equivalent.

C. elegans *germ-cells*	*n*	*DNA content (A.U.)*	*C*
Spermatocyte I (prophase)	35	260 ± 20	4*
Spermatozoa	24	53 ± 15	1

Table 3

Human lymphocyte reference * Metais & Mandel (1950), n = nuclei examined.

Cell	*n*	*DNA content (A.U.)*	*DNA* $.10^{-12}$ g
Human lymphocyte	57	2 273 ± 30	6.9 ± 0.4 *

MICRODENSITOMETRIC ANALYSIS (Tab. 1 to 3)

1) For each larval and adult stage, DNA-contents of all intestinal nuclei are the same. Variance analysis (Fischer test) indicates no significant variation between worms nor with nuclei position (anterior or posterior intestinal region) in the individual.

2) Intestinal nuclei DNA-content increases during post-embryonic development as a 2n series. Consequently there is a correlation between DNA-content and developmental stage. Comparison with *C. elegans* germ-cell DNA-content permits an estimate of the different ploidy levels. No chromatin diminution was detected by our analysis. Hence the maximum ploidy level observed is 32 haploid genome copies in the adult stage.

3) Absolute haploid DNA-contents have been investigated by comparison with human lymphocyte DNA-contents. This allowed us to estimate $0.1 \times 10^{-12} - 0.2 \times 10^{-12}$ g DNA per haploid genome in *C. elegans* (by biochemical methods, Sulston and Brenner (1974) detected a DNA-content of 0.08×10^{-12} g).

Discussion

Feulgen microdensitometric analysis clearly indicates an increase of nuclear DNA-content as a 2n series during postnatal ontogenesis of the *C. elegans* intestine. Since the number of these nuclei is constant from the second larval stage on, these non-dividing nuclei remain able to replicate their DNA by an endopolyploid process. As there is no variation in DNA-content among nuclei of one individual, this shows that intestinal nuclei endoreplicate their DNA in apparent synchrony and in correlation with the four stages of ecdysis. However, only fourteen intestinal nuclei divide during L1 to L2 ecdysis. Since all 34 nuclei retain the same ploidy level, endopolyploidization is independent of the number of previous mitotic cleavages. Then, the phenomenon is under positive genetic

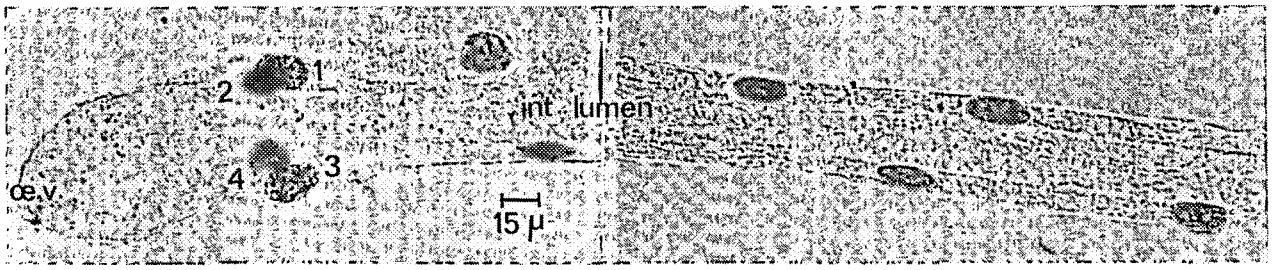

Fig. 1. *C. elegans* intestine nuclei (anterior region) with Feulgen staining procedure. 1, 2, 3, 4 : the four anterior nuclei, in.lu. : intestinal lumen ; œ.v. : oesophageal valve, × 350.

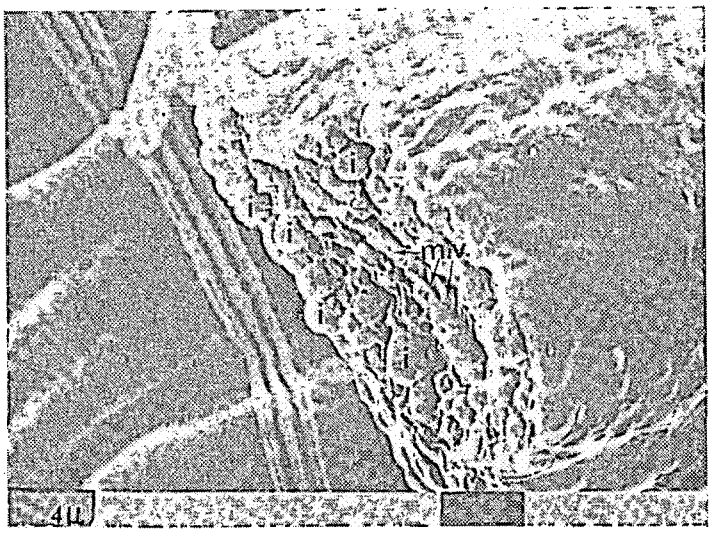

Fig. 2. *C. elegans* intestine in scanning electron microscopy (Photo from M.A. Thevenin-Gibert ; unpublished data) ; m.v. = microvilli (around the intestinal lumen) ; i = intestine inclusion, × 2 500.

control which may be altered in some dumpy mutants (Abdulkader & Brun, 1978).

Another important aspect of these observations concerns the functional significance of the endopolyploid process. Actually no single solution to this problem exists despite many investigations (Barlow, 1978 ; Brodsky, 1977 ; Nagl, 1978). However, high heterosynthetic activity appears as a common feature of endopolyploid cells, most of which have secretory functions (Anissimov 1976 ; Nagl 1978). Intestinal cytoplasm of *C. elegans* reveals many thousands of large inclusions in light and electron microscopy (Fig. 2). Since this number increases during worm development, an abundant heterosynthetic activity would be correlated with endopolyploidization.

On the other hand, intestine cell differentiation seems to occur during early embryonic development ; the eight intestinal precursor cells (E cells) present a characteristic autofluorescence (Depp *et al.*, 1978) and rhabditin is observable before hatching (Cobb, 1914). In this regard endopolyploidy seems not to be involved in intestinal differentiation processes.

ACKNOWLEDGEMENTS

We wish to thank R. Grantham, C. Souchier, M.A. Thévenin, The Quantimetry Center and C.M.E.A.B.G.

of Lyon-I for technical assistance. This work was supported by a grant from the C.N.R.S. (L.A. nº 92). The secretarial assistance of Mrs L. Fourets is gratefully acknowledged.

References

ABDULKADER, N. & BRUN, J. L. (1978). Induction, detection and isolation of temperature-sensitive lethal and/or sterile mutants in Nematodes. I. The free-living Nematode *C. elegans*. *Revue Nématol.*, 1 : 27-38.

ALTMAN, F. P. (1975). Quantitative histochemistry of some commercially available microdensitometers. *Histochem. J.*, 7 : 375-395.

ANISSIMOV, A. P. (1976). Polyploidization and growth of giant nuclei of oesophageal glands during postnatal ontogenesis in *Ascaris suum*. *Citologija (S.S.S.R.)*, 18 : 441-450.

BARLOW, P. W. (1978). Endopolyploidy, toward an understanding of its biological signifiance. *Acta Biotheor.*, 27 : 1-18.

BRENNER, S. (1974). The genetics of *C. elegans*. *Genetics*, 77 : 71-94.

BRODSKY, W. Y. (1977). Cell polyploidy : its relation to tissue growth and function. *Int. Rev. Cytol.*, 50 : 275-327.

BRUGAL, G. & CHASSERY, J. M. (1977). Un nouveau système d'analyse densitométrique et morphologique des préparations microscopiques. *Histochemistry*, 52 : 241-258.

BRUN, J. L. (1966). L'adaptation aux températures élevées chez un Nématode : *C. elegans* Maupas 1900. I. L'adaptation et son évolution. *Ann. Biol. anim. Biochim. Biophys.*, 6 : 127-158.

COBB, N. A. (1914). Rhabditin. *J. Parasitol.*, 1 : 40-41.

DELAVAULT, P. (1959). Développement et croissance des glandes génitales chez les Nématodes libres. *Archs Zool. exp. gén.*, 97 (2) : 110-205.

DEPPE, U., SCHERENBERG, E., COLET, T., KRIEG, C., SCHMITT, D., YODER, B., & EHRENSTEIN, G. von (1978). Cell lineages of the Nematode *C. elegans*. *Proc. natl. Acad. Sci. (U.S.A.)*, 75 : 376-380.

DUTT, M. K. (1976). Recent progress in staining of DNA-aldehyde in cell nuclei. *Acta Histochem.*, 56s : 120-139.

Accepté pour publication le 2 novembre 1981.

FISCHER, C. & BON, C. P. (1972). The Quantimet 720D for densitometry in the life sciences. *Microscope*, 20 : 204-211.

GABE, M. (1968). *Techniques histologiques*. Paris, Masson.

GAUTHIER, A. (1976). Ultrastructural localisation of DNA. *Int. Rev. Cytol.*, 44 : 162-163.

KIMBLE, J. & HIRSH, D. (1979). The post embryonic cell lineages in the hermaphrodite and male gonads in *C. elegans*. *Devl. Biol.*, 70 : 396-417.

KITAMOTO, Y., YAMANE, N., HOSOI, N. & ICHIKAWA, Y. (1974). Nutritional aspects of fruit-body formation of *Favolus arcularius* in replacement culture. *Trans. mycol. Soc. Japan*, 15 : 60-71.

LEUCHTENBERGER, C. (1962). Quantitative determination of DNA in cell by Feulgen microspectrophotometry. In : Altman, R. G. & Brocker, F. (Eds) *General cytochemical methods*. New York, Acad. Press : 1-148.

METAIS, P. & MANDEL, P. (1950). Teneur en acide désoxypentose l nucléique des leucocytes chez l'homme normal et à l'état pathologique. *C.R. Soc. Biol. Fr.*, 149 : 277-279.

NAGL, W. (1978). *Endopolyploidy and polyteny in differentiation and evolution*. Amsterdam, North Holland Publish, 185 p.

NIGON, V. (1945). Modalité de reproduction et le déterminisme du sexe chez quelques Nématodes libres. *Ann. Sci. nat. Zool.*, 2e série : 1-132.

PATEL, T. R. & Mac FADDEN, B. A. (1978). Axenic and synchronous cultures of *C. elegans*. *Nematologica*, 24 : 51-62.

SIN, W. C. & PASTERNACK J. (1970). Number and DNA content of nuclei in free-living Nematode *P. silusiae* at each stage during post embryonic development. *Chromosoma*, 32 : 191-204.

SINGH, R. N. & SULSTON J. E. (1978). Some observation on moulting in *C. elegans*. *Nematologica*, 88 : 63-71.

SULSTON, J. E. & HORVITZ, H. R. (1978). Post embryonic cell lineages of the Nematode *C. elegans*. *Devl. Biol.*, 56 : 27-38.

THOMAS, C. & BROWN, D., (1976). Localization of the genes for silkfibroin in silkgland cells of *B. mori*. *Devl. Biol.*, 49 : 89-100.

VANFLETEREN, J. R. (1978). Axenic culture of free-living, plant-parasitic and insect parasitic Nematodes. *A. Rev. Phytopath.*, 16 : 131-157.