Histol Histopath (1986) 1: 197-201

Histology and Histopathology

Morphological differentiation of mitochondria in the early chick embryo: a stereological analysis

P. de Paz[1], A. Zapata[2], J. Renau-Piqueras[3] and F. Miragal1[3]

[1]Department of Electron Microscopy, University of Leon, Leon, Spain;
'Department of Microscopic Morphology, Faculty of Biology, Leon, Spain;
[3]"Instituto de Investigaciones Citologicas", Valencia, Spain

Summary. The morphological evolution of mitochondria in three cell types of chick embryo in neurulation was analyzed by stereological methods. Mitochondria, showing a random distribution, were characterized by moderate electron-dense matrices and normal cristae. The numerical density of mitochondria significantly increased in the neuroectoderm and epiblastic cells while their volume density remained unchanged. The mitochondria in mesoderm cells were ellipsoidal (axial ratio 2:1) at stages 5 and 8 although they underwent an elongation in neuroectoderm and epiblastic cells (axial ratio from 2:1 to 1.6:1). The individual size of "average mitochondria" in the mesoderm cells was smaller than in other cell types. The total V/S (volume/surface) ratio of mitochondria decreased during neurulation. These morphological changes have been discussed emphasizing the possible metabolical role of mitochondria during morphogenesis.

Key words: Mitochondria - Chick embryo - Neurulation - Stereology

Introduction

Embryonic development has been divided into a morphogenetic phase, during which cells change shape or position molding in rough the tissues and organs, and a cytodifferentiation phase, during which the individual cells organize the special cellular and chemical features that characterize each particular histological state. However, a kind of morphogenetic cytodifferentiation is necessary at the beginning in order for cells to engage in the changes in shape and translocations that dominate the morphogenetic phase (Trinkaus, 1976). Thus, the cell differentiation which is initiated during the early phase of amphibian embryonic development, includes morphological variations of the mitochondria. A number of observations suggest that this morphological differentiation is associated with a differentiation in the chemical activities of this organelle (Nelson et al., 1982). In this regard, a change in volume density of mitochondria in early chick embryo has been reported by Mathieu and Messier (1976), and Paz et al. (1985). However, there is no information about possible morphological changes in mitochondria in the course of neurulation. The aim of the present paper is to analyze, by stereological methods, the changes affecting the mitochondria1 morphology of early chick embryo.

Offprint requests to: Dr. Paulino de Paz, Servicio de Microscopia Electrónica, Facultad de Veterinaria. Universidad de Leon. Leon. Spain

Materials and methods

The midbrain areas from chick embryo of stage 5 and 8 (Hamburger and Hamilton, 1951) were dissected. The specimens were fixed directly from the yolk with a 2% glutaraldehyde solution, buffered with 0.1 M cacodylate (330-360 mOs) at pH 7.4 for 1 h at 4° C, and rinsed twice in this buffer. Later, they were postfixed with a buffered osmium tetroxide solution (1%) for 3 h at room temperature and washed in buffer. Dehydration was accomplished using a graded series of ethanol solutions. The samples were embedded in Epon 812 resin. Semithin sections were stained with toluidine blue (0.5%). Ultrathin sections were mounted on 200 mesh grids and contrasted with uranyl acetate and lead citrate. They were examined using a Jeol 100 CX electron microscope at 60 kV.

Sampling procedures

Since the epiblast and neuroectoderm are polarized tissues, we only used sections cut perpendicularly to their surface (Weibel, 1979). The need for and effective random sampling when only a small proportion of the available material can be examined at ultrastructural level has been described by Weibel et al. (1969). Thus, according to general procedures described by Williams (1977), we used the following steps. Thirty embryos (midbrain area according to Schoenwolf and Franks, 1984) of each stage were used. Five blocks were selected from each stage by digital random table. Five ultrathin sections were obtained perpendicularly to the cephalocaudal axis from each selected block and mounted on 200 mesh grids. The pool of grids was observed at x 8,300. Thus, 12 micrographs were obtained in the upper left corner of consecutive squares of each grid. The calibration was performed using standard calibration grid. Each embryonic layer was separately sampled on each section. Micrographs were printed at x 18,000. The minimum sample size was determined using the progressive mean technique in all cellular types (Williams, 1977).

Stereological procedure

The micrographs were analyzed by planimetry with a Leitz ASM system for a semiautomatic image analysis. The outlines of all mitochondria were traced by electronic pen. A range of 75-80 cells from each embryonic layer was analyzed per stage.

The volume density of mitochondria was obtained according to Weibel and Bolender (1973) $Vvm = Ai/At$, where **Ai** = total area of mitochondria profiles and **At** = area of cytoplasm. The surface density of mitochondria was calculated in the same way ($Svm = Lm/At$, where **Lm** = perimeter of mitochondria profiles). The coefficient form was determined according to the expression $CF = 4\pi$ area/(perimeter)^2 (Nelson at al., 1982). The numerical density of mitochondria was obtained as $Nvm = K/b.\ Na^{3/2}/Vv^{1/2}$ (**Na** = number of mitochondria profiles per unit area of cytoplasm and **Vv** = particle volume density) (Weibel and Gomez, 1962). The mitochondria was considered as an ellipsoidal particle with a factor K = 1.50 (the particles have not the same size) and a constant b = 1.78 (mean axial ratio = 2.5:1), values calculated according to Williams (1977). In this sense, White and Gohari (1983) used other calculations of numerical density assuming that the mitochondria were spherical particles and generated circular profiles on sections. They considered Nv estimates, using the method of Weibel and Gomez (1962), as' probably the most accurate and therefore the most representative.

It is possible to calculate the volume or number of mitochondria present in the "average cell" (White and Gohari, 1983) after calculation of the parameters Vv and Nv. The methods for estimating cytoplasmic volume (Vcy) are based on determnation of nuclear-cytopfasmic ratio by planimetry and evaluation of nuclear volume by direct measurement (Petrzilka et al., 1978). The mean volume (Vm'), surface (Sm') and number (Nm') of mitochondria present in the "average cell" from each cellular layer were calculated according to the relationships: $Vm' = Vm \times Vcy$, $Sm' = Svm \times Vcy$ and $Nm' = Nvm \times Vcy$. The mean volume (Vm) and surface (Sm) of "average mitochondria" were obtained by dividing: Vm'/Nm' and Sm'/Nm', respectively.

The mean (± standard deviation) was calculated for each parameter. The variation between different stages was analyzed in each embryonary layer to obtain the F value. When a significant F was found, Duncan's new multiple range test was used to compare the means.

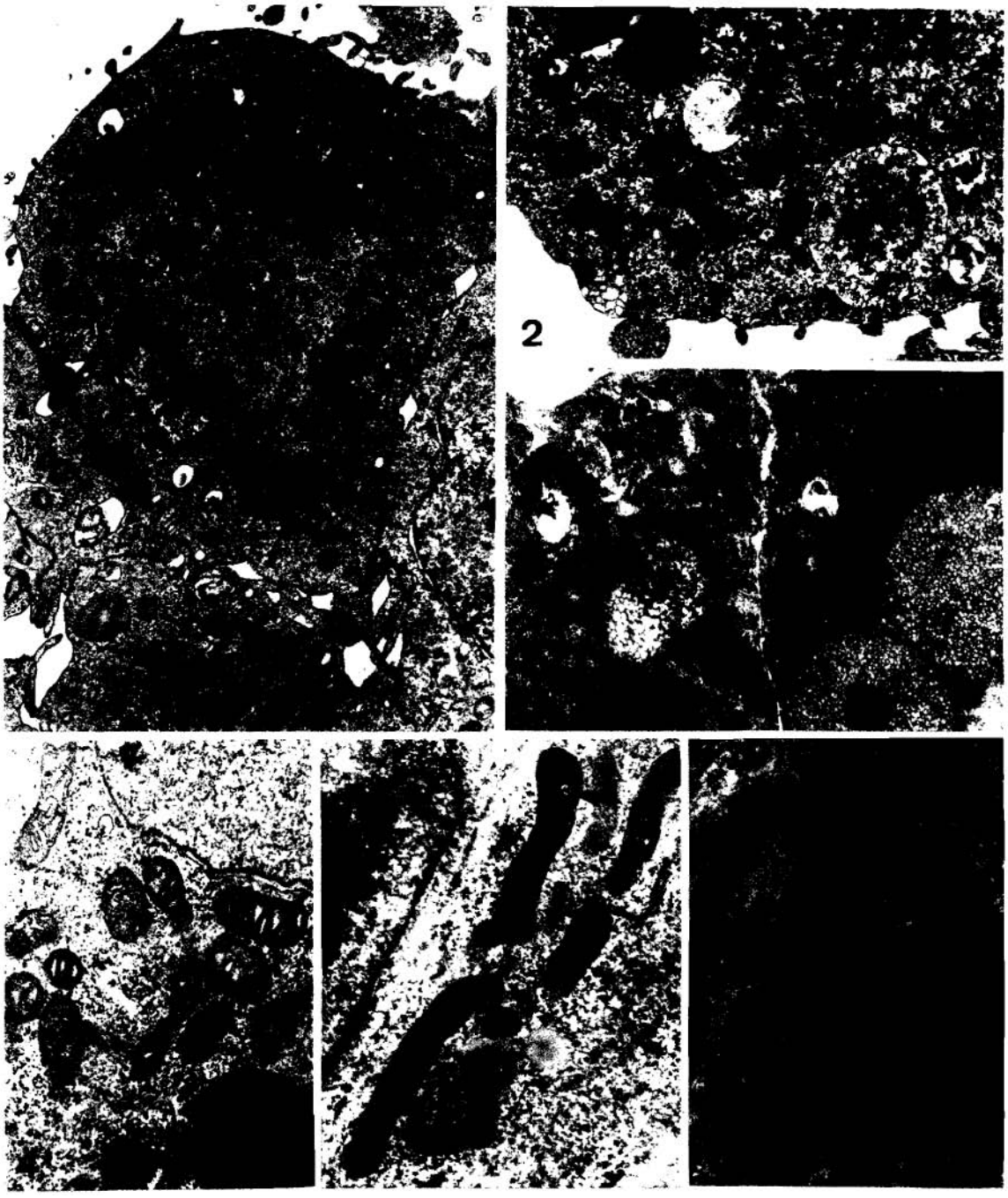
Results

The mitochondria in the three cellular types have no special location on the cell section (Fig. 1). Mitochondria1 profiles were generally elliptical although circular profiles were also found. The mitochondrial matrices were generally electrondense with some electrolucide areas (Figs. 1, 5) and the cristae appeared occasionally dilated (Fig. 4).

The volume density of mitochondria (Vvm) (percentage of cytoplasm filled by the mitochondria) remained constant from stage 5 to 8 in the three cell types. Both stages, the mesodermic cells contained a higher amount of mitochondrial volume (Table 1). The surface density of mitochondria (pm^2 external membrane of mitochondria/μm^3 cytoplasm) did not significantly change in the cell types studied (mean value 0.828 $\mu m^2/\mu m^3$). The numerical density of mitochondria (Nvm) (number of mitochondria/μm^3 in the cytoplasm) in stage 5 mesoderm cells was higher than that of the neuroectoderm and epiblast cells. The two latter cellular types increased their numerical densities from stage 5 to 8 (75.6% and 64.2% respectively) reaching greater values than those of the mesoderm cells (Table 2).

The volume of the "average mitochondria" (Vm) in mesoderm cells was smaller (0.086 μm^3) than that observed in the neuroectoderm (0.122 μm^3) and epiblast (0.132 μm^3) cell at stage 5. This volume decreased significantly during neurulation (neuroectoderm 33.61%, epiblast 44.69% and mesoderm 25.47%, see Table 2). The mean surface of mitochondria1 membrane (Sm) diminished in this period by 38.07% in neuroectoderm cells, 39.48% in epiblast cells and 19.24% in mesoderm cells. The V/S (Volume/surface) values showed significant differences between the three cellular types at each stage (Table 2).

The axial ratio of mitochondria only showed significant differences between the various cell types at stage 8. The mitochondria in the neuroectoderm and epiblast cells became more elongated (12.90% and 10.90% respectively, see Figs. 1, 3, 5 and 6) and ellipsoidal than those in the mesoderm cells (Figs. 2, 4). Similar differences were found related to the coefficent form (Table 2).



Figs. 1-6. Mitochondria in three types of early chick embryo in neurulation. **Stage** 5: **Fig. 1.** Epiblast cell showing a random location of mitochondria (arrowhead). Areas of electrolucide matrix could be observed (arrows). x 6,200. **Fig. 2.** Mesoderm cell. x 12,750. **Fig. 3.** Neuroectoderm cell. × 10,500. **Stage 8: Fig. 4.** Mesoderm cell with some mitochondria showing remarkable dilatations of some cristae. x 18,250. **Fig. 5.** Epiblast cell. Arrows indicate electrolucide matrix areas. × 17,550. **Fig. 6.** Neuroectoderm cells. These last two figures show mitochondria that present a characteristic change in shape with respect to identical mitochondria at stage 5 (Figs. 1, **3).** × 16,750.

Table **1.** General stereological parameters of mitochondria in three cellular types at stages-5 and 8 (mean + standard deviation).

CELLULAR TYPE	PARAMETERS							
	Vvm ($\mu m^3/\mu m^3$)		Svm ($\mu m^2/\mu m^3$)		Nvm ($n^o/\mu m^3$)		n	
	st. 5	st. 8	st. 5	st. 8	st. 5	st. 8	st. 5	st. 8
Neuroectoderm	^{a}0.0578 (.0261)	**0.0590 (0.0254)**	**0.836 (0.297)**	**0.884 10.361)**	^{a}0.501 (0.293)^{x}	**0.880** (0.380)^{y}	**530**	**680**
Mesoderm	^{b}**0.0778 (0.0676)**	**0.0676 (0.0253)**	**0.842 (0.282)**	**0.880 (0.289)**	^{b}**0.744** (0.143)	**0.739** (0.253)	**590**	**610**
Epiblast	**08.0527 (0.0199)**	**0.0534 (0.0234)**	**0.738 (0.269)**	**0.790** (0.217)	^{a}0.500 (0.091)^{x}	**0.821** (0.109)^{y}	**510**	**585**

Different letters (a,b,) show significant differences ($p < 0.05$) between cellular types at each stage
Different letters (x,y) show significant differences ($p < 0.05$) between stages for each cell type
n = Number of analyzed mitochondria
Vvrn* = Density of mitochondrial volume
Svrn = Density of mitochondrial surface (external membrane)
Nvrn* = Density of mitochondria number
(*) reference area = cytoplasm

Table **2.** Individual stereological parameters (mean ± standard deviation) of "average mitochondria" in three cellular types of early chick embryo (stage 5 and 8).

CELLULAR TYPE	PARAMETERS									
	Vm (μm^3)		Sm (μm^2)		V/S (μm)		CFm (—)		Axial ratio	
	st. 5	st. 8	st. 5	st. 8	st. 5	st. 8	st. 5	st. 8	st. 5	st. 8
Neuroect.	^{a}0.122 (0.044)^{x}	**0.081** (0.034)^{y}	**1.590** (0.420)^{x}	**1.080** (0.380)^{y}	^{a}0.076	^{a}0.075	**0.806** (0.044)^{x}	^{a}0.702 (0.041)^{y}	**2.03** (0.21)^{x}	^{a}2.76 (0.27)^{y}
Mesoderm	^{b}0.086 (0.034)^{x}	**0.064** (0.023)^{y}	**1.301 (0.41)**^{x}	**1.044** (0.328)^{y}	^{b}**0.066**	^{b}**0.061**	**0.817** (0.041)	^{b}**0.831** (0.066)	**1.93 (0.141**	^{b}1.82 (0.23)
Epiblast	^{a}0.132 (0.059)^{x}	**0.073** (0.025)^{y}	**1.547** (0.562)^{x}	**0.932** (0.223)^{y}	^{a}0.085	^{a}0.078	**0.800** (0.034)^{x}	^{a}0.713 (0.072)^{y}	**2.01** (0.26)^{x}	^{a}2.63 (0.30)^{y}

For significant differences, see Table 1
Vrn = Volume of "average mitochondria"
Sm = Surface of "average mitochondria" (external membrane)
V/S = Volume/Surface ratio of "average mitochondria"
CFm = Coeffient of mitochondria form
Axial ratio = Axis major/axis minor

Discussion

The cellular differentiation initiated during early phases of embryonic development (morphogenetic phase) includes morphological alterations of mitochondria. These variations involve the number, size, shape and intracellular localization of the mitochondria and clearly appear to be related to the specific functions of the tissue (Ernster and Schatz, 1981).

Each population of mitochondria possessing a high heterogeneity (David, 1975) could explain the high coefficient of variation (standar deviation expressed as a percentage of the mean) here observed. The mitochondrial volume of the mesoderm cells decreased by 68% during neurulation and a reduction in size of "average mitochondria" (25.03%) and V/S ratio (7.03%) was also observed. The volume of "average mitochondria" in the neuroectoderm and epiblast cells decreased in this period (33.6% neuroectoderm and 44.7% epiblast) although an increase in numerical density (75.6% and 64.2% respectively) was also observed. In this regard, David (1975) emphasized that an increase in the number of mitochondria could be correlated with a simultaneous reduction in the volume of each mitochondrion. These transformations could also be analyzed by taking into account the changes these cells undergo during the neurulation process which implies a high energetic comsumption. Thus, in this period, epiblast cells undergo an expansive behaviour including a high multiplication index and changes in cellular shape (Downie, 1976). Likewise, neuroectoderm cells show changes in shape as a basic mechanism for neurulation (Jacobson, 1981;

Shoenwolf and Franks, 1984). Finally, mesoderm cells exhibit a characteristic migratory behaviour (Ebendal, 1976; Solursh and Revel, 1978) which also requires an active metabolism (Kamimura et al., 1980). In this regard, the higher number and volume densities of mitochondria in mesoderm cells at stage 5 could be related to the above-mentioned active metabolism.

On the other hand, stage 5 mitochondria show an axial ratio aproximately 2:1 in three cellular types. At stage 8 they change remarkably; thus, mesoderm cells have ellipsoidal mitochondria and neuroectoderm and epiblast cells show more elongated mitochondria (axial ratio 2.6:1). This mitochondrial elongation has also been described in early amphibian embryos (Nelson et al., 1982) when the undifferentiated embryonic cells are undergoing differentiation. However, White and Gohari (1983) observed more spherical mitochondria during the epithelial defferentiation in the cheek pouch mucosa of hamsters.

Although the relationship between our own quantitative results on mitochondrial morphology and the biochemical studies about its respiratory activity are difficult, the relationship between mitochondrial configuration and its metabolic state is widely accepted (Bereither-Hahn and Voth, 1983). Cech and Sedlackova (1983) analyzing the volume density of mitochondria in mouse blastodiscs correlated the changes in form and internal structure of this organelle with disturbances in its energetic role and/or biogenesis. Therefore, it is possible that the alterations in the number, size and shape of mitochondria here described might reflect main changes in the respiratory cell mechanism related to the variations of cell metabolism during differentiation (White and Gohari, 1983).

References

Bereither-Hahn J. and Voth M. (1983). Metabolic control of shape and structure of mitochondria in situ. Biol. Cell 47, 309-332.

Cech S. and Sedlackova M. (1983). Ultrastructure and morphometric analysis of preimplantation mouse embryos. Cell. Tissue Res. 230, 661-670.

David H. (1975). Quantitative morphology of mitochondria. Biol. Zentr. 94, 129-153.

Downie J.R. (1976). The mechanism of chick blastoderm expansion. J. Embryol. Exp. Morph. 35, 559-575.

Ebendal T. (1976). Migratory mesoblast cells in the young chick embryos examined by SEM. Zoon 4, 101-108.

Ernster L. and Schatz G. (1981). Mitochondria: a historical review. J. Cell Biol. 91, 227s-255s.

Hamburger V. and Hamilton H.L. (1951). A series of normal stages in the development of the chick embryos. J. Morph. 88, 49-92.

Jacobson A.G. (1981). Morphogenesis of the neural plate and tube. In: Morphogenesis and pattern formation. Connelly T.G. (ed). Raven Press. New York.pp 233-263

Kamimura M., Kotani M. and Yamagata K. (1980). The migration of presumptive primordial germ mass trought the endodermal mass in Xenopus *laevis:* A light and electron microscopic study. J. Embryol. Exp. Morph. 59, 1-17.

Mathieu O. and Messier P.E. (1976). Stereological studies of the neural organogenesis in the chick embryo. In: Fourth Inter. Cong. Stereol. (ed. E.E. Underwood, R. de wit and G.A. Moore).pp 393-396.

Nelson L., Lonrentzon R., Boquist L. and Lovtrup S. (1982). Morphological differentiation of mitochondria in the early amphibian embryo. Exp. Cell Res. 137, 25-29.

Paz P., Zapata A., Fernández J.G., Chamorro C. and Villar J.M. (1985). Evidence of non-early ultrastructural regionalization in the neural epithelium by stereological methods. Acta Anat. 124, 227-233.

Petrzilka G.E., Graaf de Beer M. and Schroeder H.E. (1978). Stereological model for the cells and based-line data for human peripheral blood derived small T lymphocytes. Cell Tissue Res. 197, 121-142.

Schoenwolf G.C. and Franks M.V. (1984). Quantitative analysis of changes in cell shapes during bending of the avian neural plate. Dev. Biol. 105, 257-272.

Solursh M. and Revel P.J. (1978). A scanning electron microscopy study of cell shape and cell appendages in the primitive strak region of the rat and chick embryo. Differentiation 11, 185-190.

Trinkaus J.P. (1976). On the mechanism of metazoan cell movement. In: Cell surface reviews, vol. 1, The cell surface in animal embryogenesis and development. Poste G. and Nicolson G. L. (eds). North-Holland Publishing Company, Amsterdam.

Weibel E.R. (1979). Stereological methods, vol. 1, Practical methods for biological morphometry. Academic Press, London.

Weibel E.R. and Gomez D.M. (1962). A principle for counting tissue structures on random sections. J. Appl. Physiol. 17, 343-348.

Weibel E.R. and Bolender R.P. (1973). Stereological techniques for electron microscopy morphometry. In: Principles and techniques in electron microscopy. Hayat M.A. (ed). Van Norstrand Reinhold Co. New York. pp 239-283.

Weibel E.R., Staubli W., Gnagi H.R. and Hess F.A. (1969). Correlated morphometric and biochemical studies on the liver cell. I. Morphometric model, stereologic methods and normal morphometric data for rat liver. J. Cell Biol. 42, 68-91.

White F.H. and Gohari K. (1983). Stereological studies on differentiation in hamster cheek pouch epithelium: variations in the volume and frequency of mitochondria. J. Anat. 136, 601-619.

Williams M. (1977). Stereological techniques. In: Practical methods in electron microscopy. Glauert A.M. (ed). North-Holland Amsterdam. pp 5-84.

Accepted December 7, 1985