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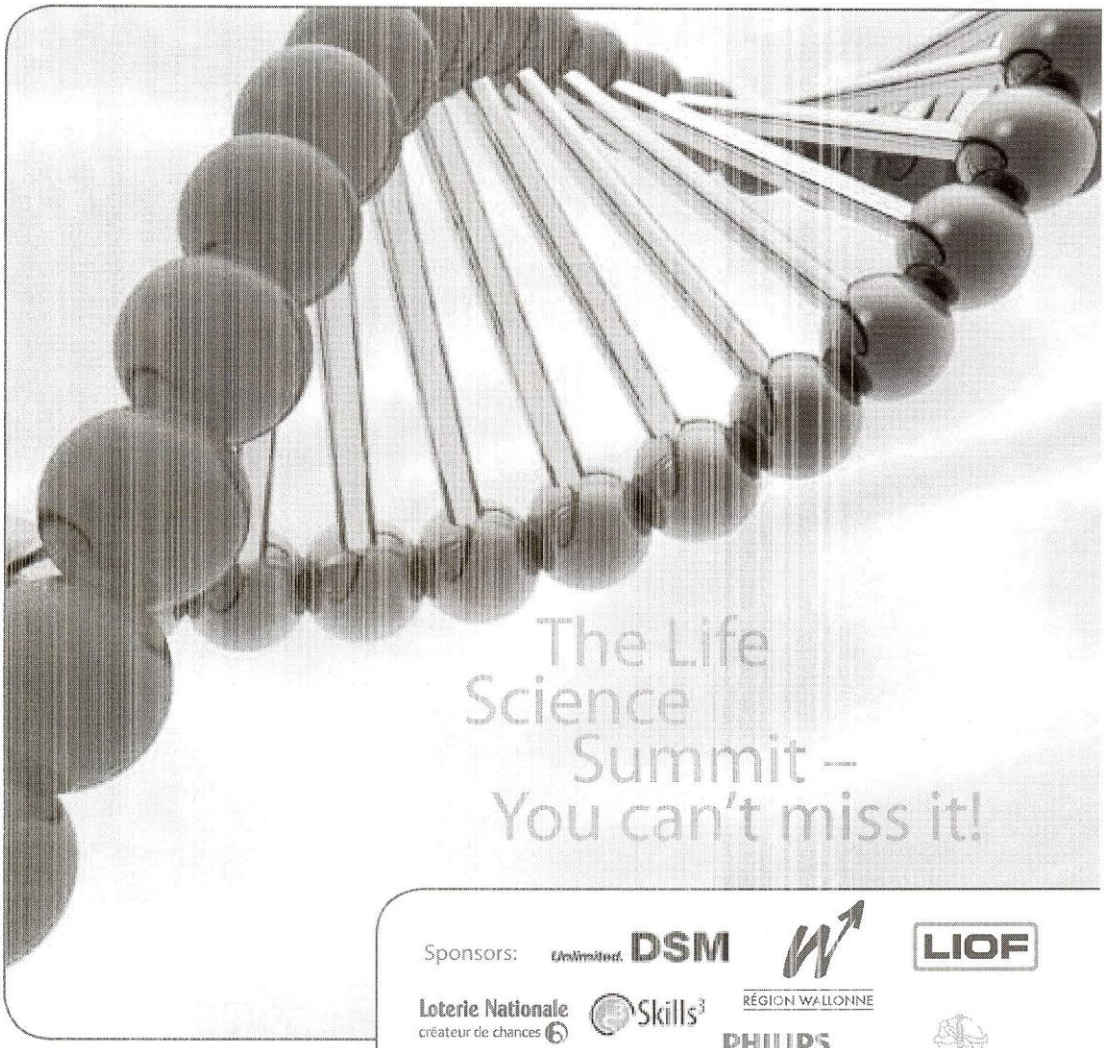

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Turtle cell cultures for nucleolar studies

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Recently, an exhaustive statement of literature about the fine structure of the nucleolus in vertebrate cells has revealed that in anamiotes the nucleolus consisted of two major compartments whereas in amniotes it was composed of three main compartments. Although the precise structure of the nucleolus was well-known in mammals and birds, it had only been described in two reptilian species.

During the two last years, we have investigated the ultrastructural organization of the nucleolus in different species belonging to four groups of reptiles (lizard, snake, turtle and crocodile). Our results clearly indicated that both nucleolar types were present in reptiles: a bipartite nucleolus in turtles and in snakes and a tripartite nucleolus in lizards and in crocodiles, suggesting that the tripartite nucleolus should be appeared in some reptiles. These observations were realized on various tissue samples taken from the different reptilian species and thereby required sacrifice of some animals.

To complete our previous investigations on the architecture of the nucleolus in reptiles and in particular on the functional organization of bipartite nucleolus, we have developed in vitro cell cultures from tissues of an aquatic turtle *Trachemys scripta scripta*. Cells were extracted and cultured from liver and spleen of this turtle. Homogeneous cultures were obtained by repeatedly forcing minced, frequently washed tissue through a sterile screen and separating the resulting cells by centrifugation. Both cells were maintained in culture for over 5 weeks in RPMI 1640-based medium supplemented with foetal bovine serum. They attached to the T flask substrate as individual cells and aggregates and spread out 14 days after being placed in media. The cells can be frozen and defrosted.

Cells from spleen were characterized by many cytoplasmic processes whereas cells from liver were spindle-shaped. Both cells exhibited a large nucleus with one or two well obvious nucleoli. On ultrathin sections, the cell nucleolus was composed of two compartments: a centrally-located, fibrillar zone surrounded by a granular zone. In the fibrillar zone, a cordon of densely-contrasted material was further observed. When cells were cultured in presence of a low concentration of actinomycin D, a preferential inhibitor of ribosomal RNA synthesis, the nucleoli segregated. However, the electron dense material condensed and remained in the central part of the segregated fibrillar zone. This compacted material also contained DNA as revealed with the terminal deoxynucleotidyl transferase-immunogold labelling procedure.

These turtle cell cultures open new perspectives for the study of the functional organization of the nucleolus in reptiles.