REPTILE 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 (Thiry and Lafontaine, 2005, TCB 15:194-199) has revealed that in anamiotes the nucleolus consisted of two major compartments (a fibrillar zone surrounding by a granular zone) whereas in amniotes it was composed of three main compartments (the fibrillar center, the dense fibrillar component and the granular component). Although the precise structure of the nucleolus was well-known in mammals and birds, it had only been described in two lizard 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. It is also interesting to note that all the tissues from a same species had the same type of nucleolus, although the importance and the repartition of those components can vary from a tissue to another one. Curiously, in bipartite nucleoli, we also showed that the fibrillar zone was not homogeneous but contained densely-contrasted, fibrillar cordons. Both, DNA and AgNOR proteins were preferentially detected in these cordons. In tripartite nucleoli, AgNOR proteins were observed in the fibrillar centres and in the dense fibrillar component but DNA was only significantly detected in the fibrillar centres.

To better understand the functional organization of the nucleolus in reptiles, and in particular in bipartite nucleolus, we developed in vitro cell cultures from tissues (liver and spleen) of an aquatic turtle *Trachemys scripta scripta.* A lung epithelial cell culture from a lizard *Gekko gecko* was used as control of tripartite nucleolus. Under these *in vitro* conditions, the nucleolar compartmentalization was identical to that observed in tissue samples. When ribosomal RNA synthesis was preferentially inhibited by a treatment with actinomycin D, we observed in tripartite nucleoli, a classical segregation of three main nucleolar components. By contrast, in bipartite nucleoli, we showed that the electron-dense cordons of the fibrillar zone were concentrated in the centre of segregated fibrillar zone.

These results suggest that both distinct fibrillar components in the tripartite nucleolus would come from a redistribution of components present in the fibrillar zone of the bipartite nucleolus.