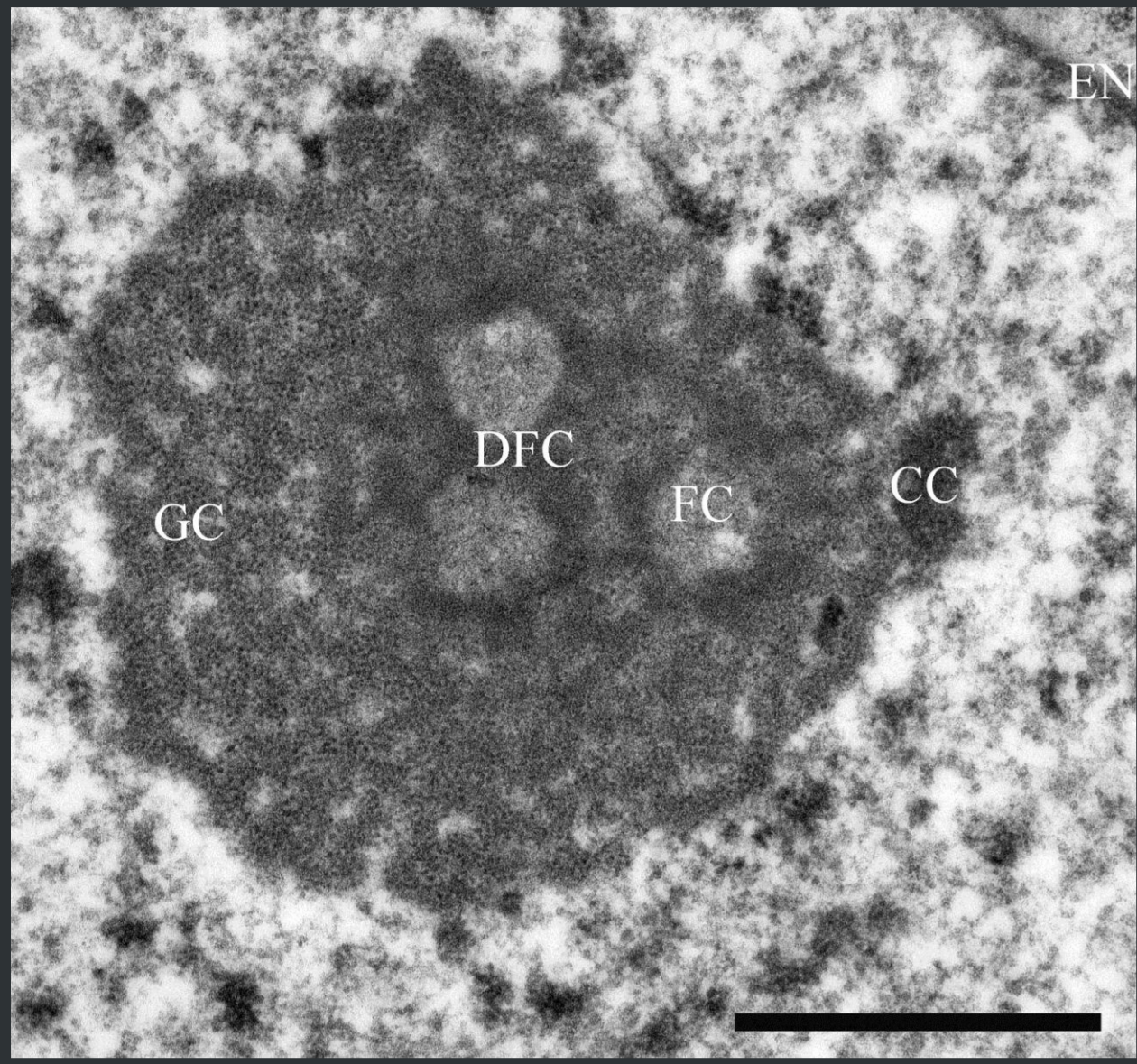


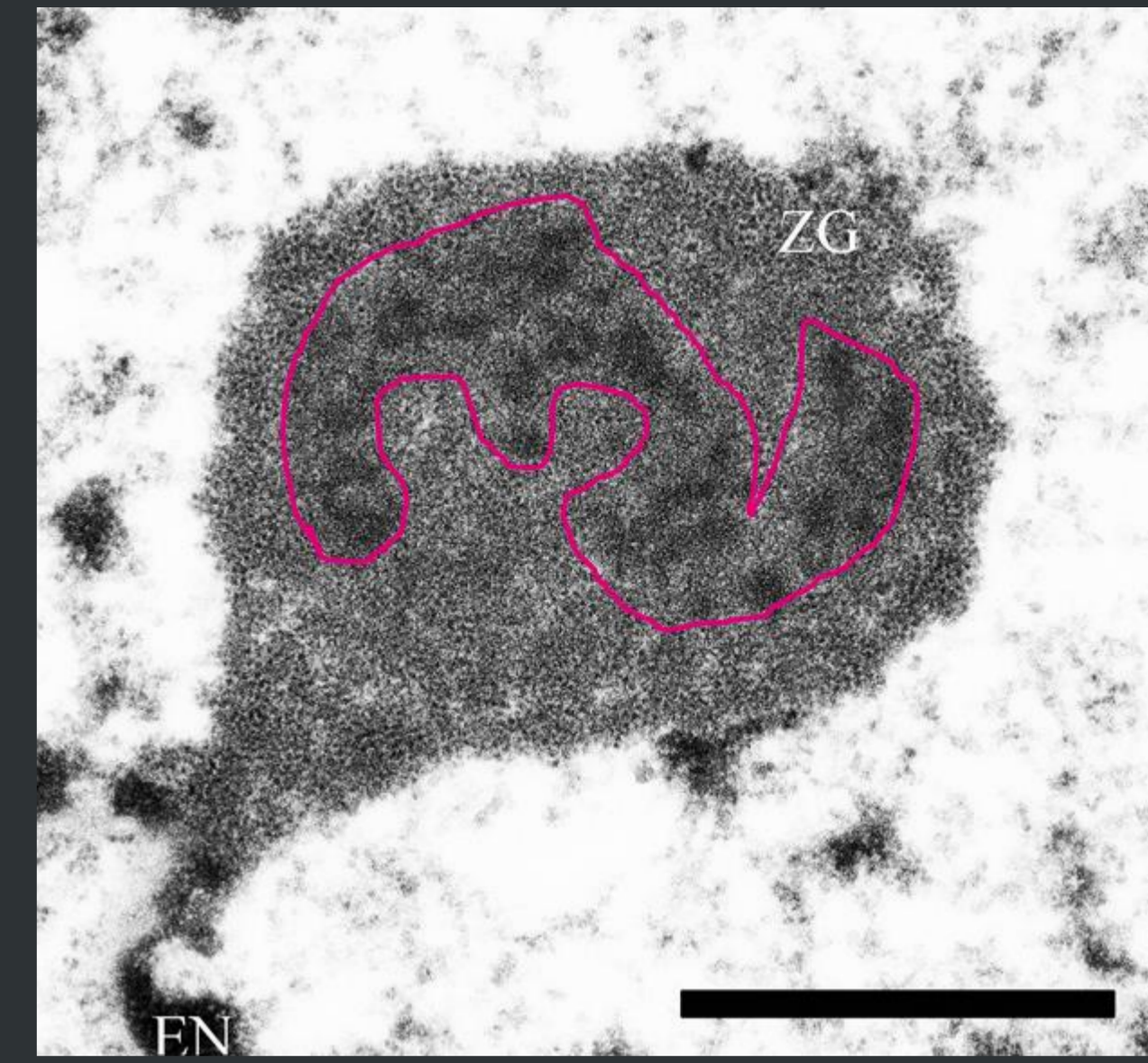
Introduction



1. Lizard nucleolus from epithelial cell. FC: fibrillar center; DFC: dense fibrillar component; GC: granular component. Bar: 1 μ m.

The nucleolus is the most distinct structure of the nucleus in eukaryotic cells. The main function of the nucleolus is ribogenesis and it is generally assumed that each structural compartment of the nucleolus is associated with a step of this process, demonstrating a strong structure/function relationship in the nucleolus.

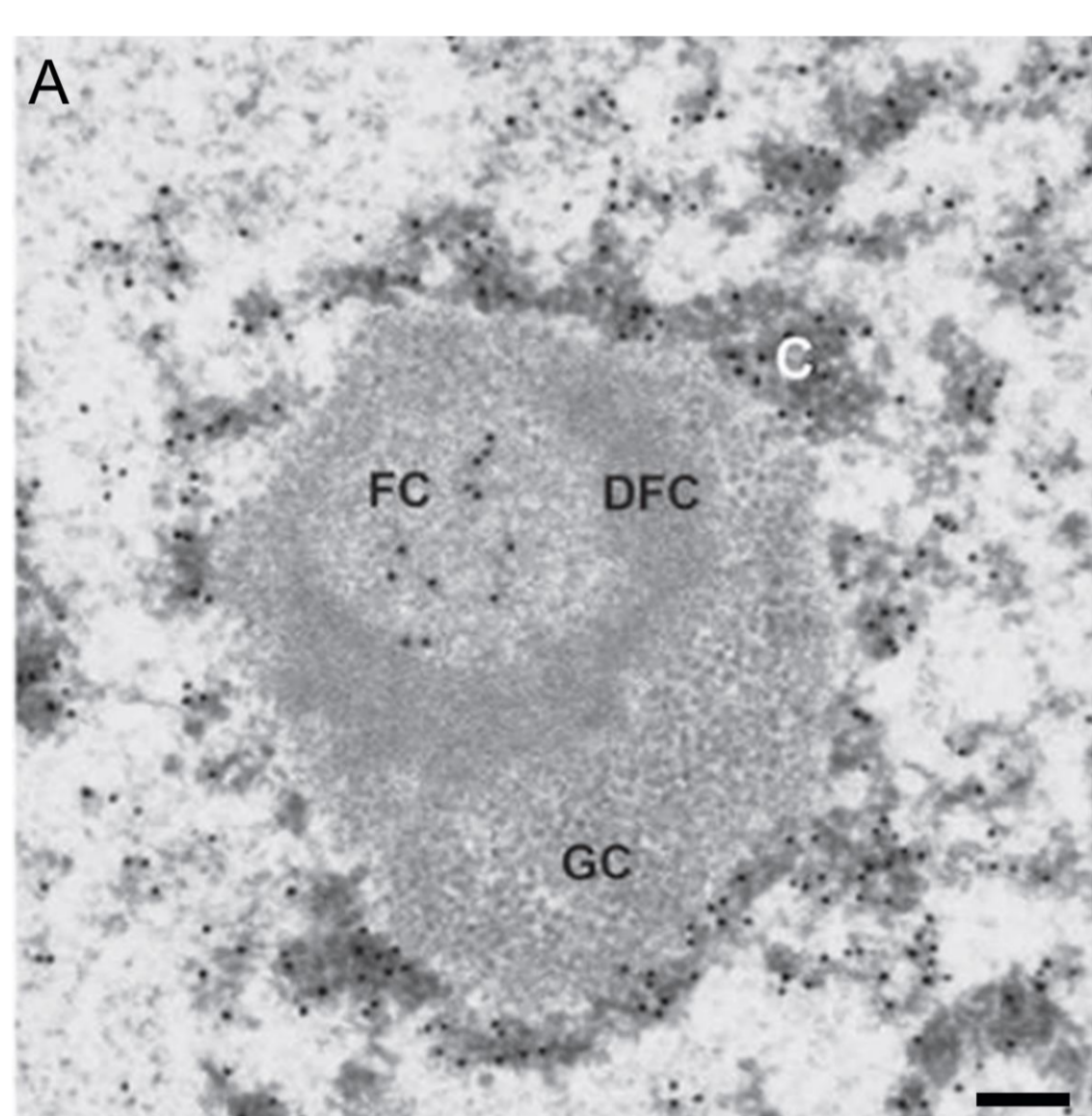
This process has been mostly conserved during evolution; however two different nucleolar organizations can be observed: most amniotes, including humans, display a nucleolus with three different compartments (Figure 1) while the rest of the eukaryotes present a nucleolus with only two compartments (Figure 2). The first type of nucleolus has already been studied in-depth however the second remains more enigmatic.



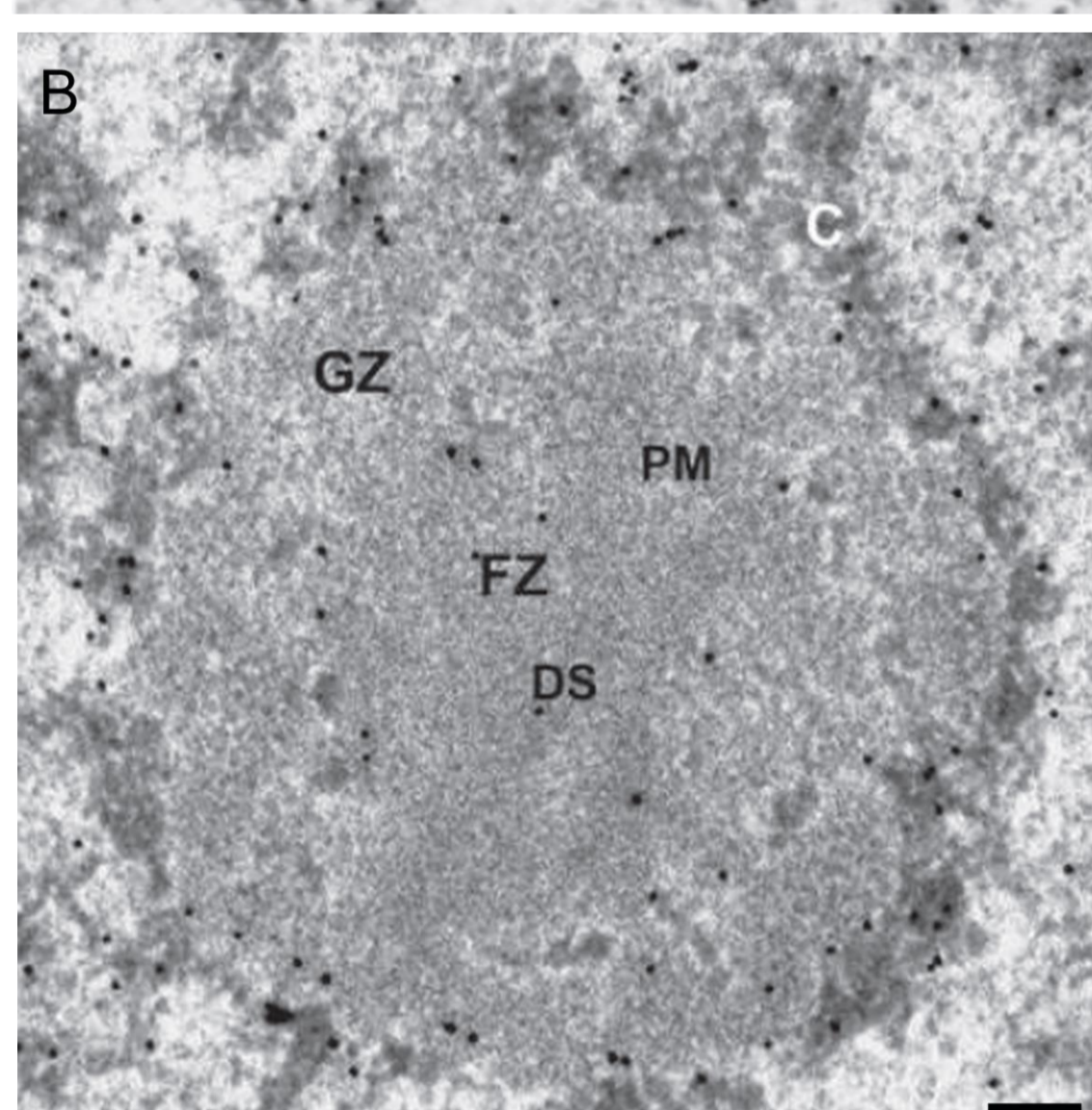
2. Turtle nucleolus from hepatocyte. Pink area: fibrillar zone; GZ: granular zone. Bar: 1 μ m.

Results

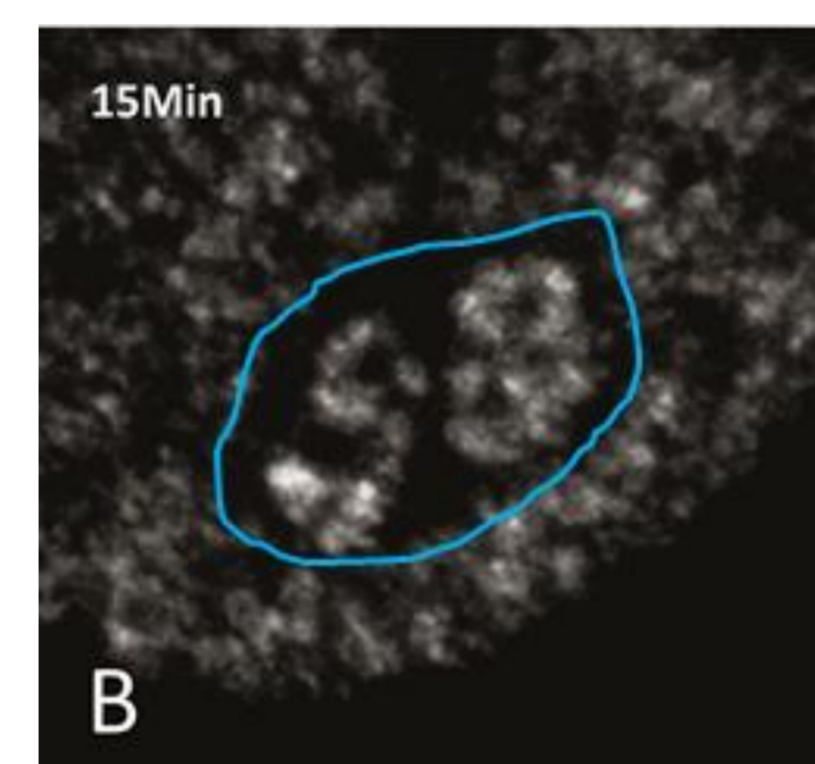
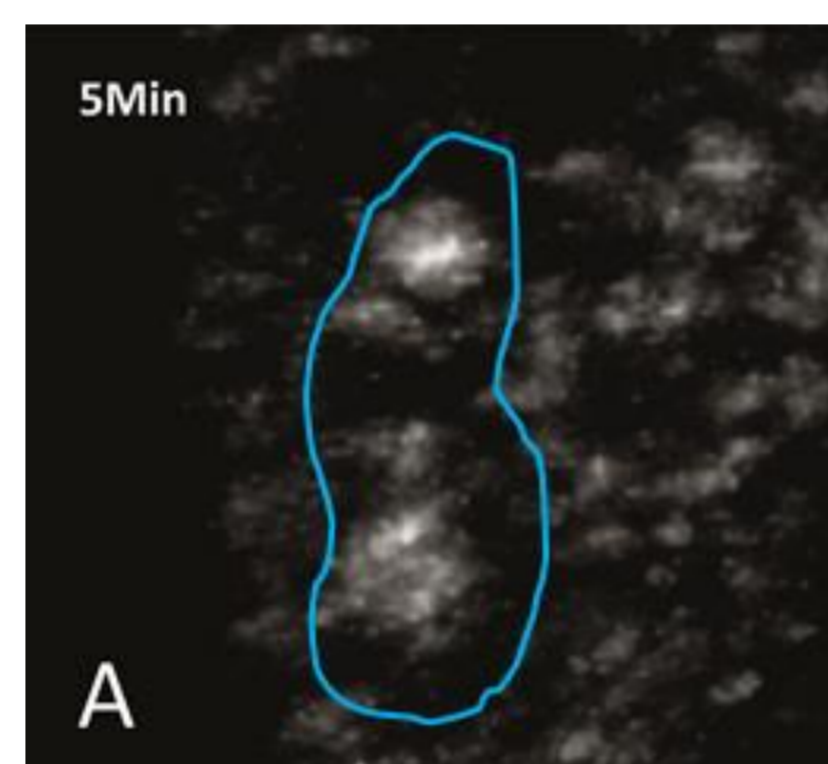
To better understand the relationships between structure and function in these two types of nucleoli, we investigated the location of the rRNA synthesis site within the nucleolus of cultured cells from two reptilian species: a turtle with a bicompartimentalized nucleolus and a lizard with a tricompartmentalized one.



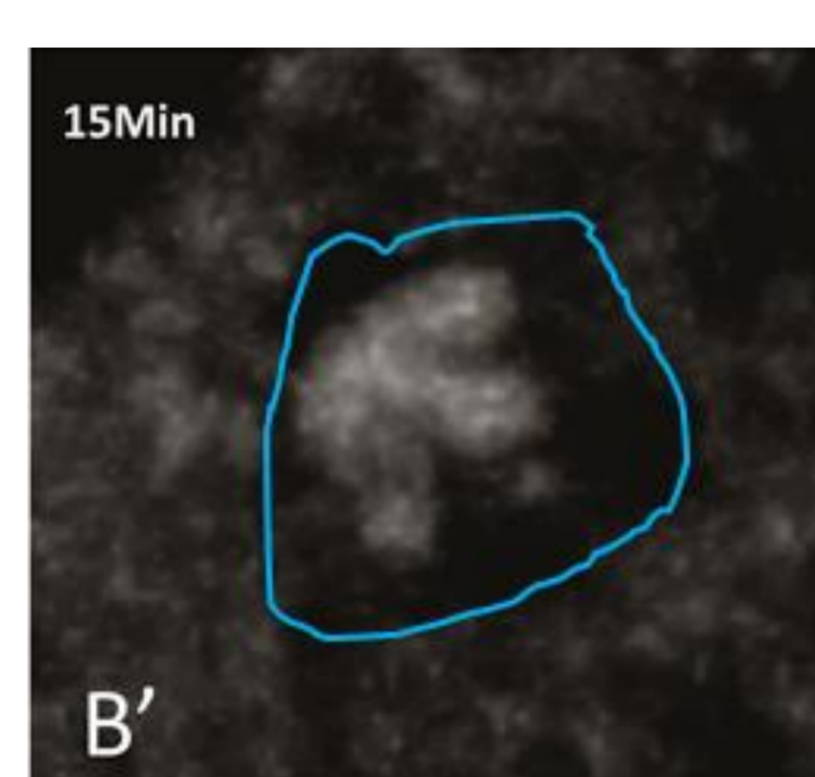
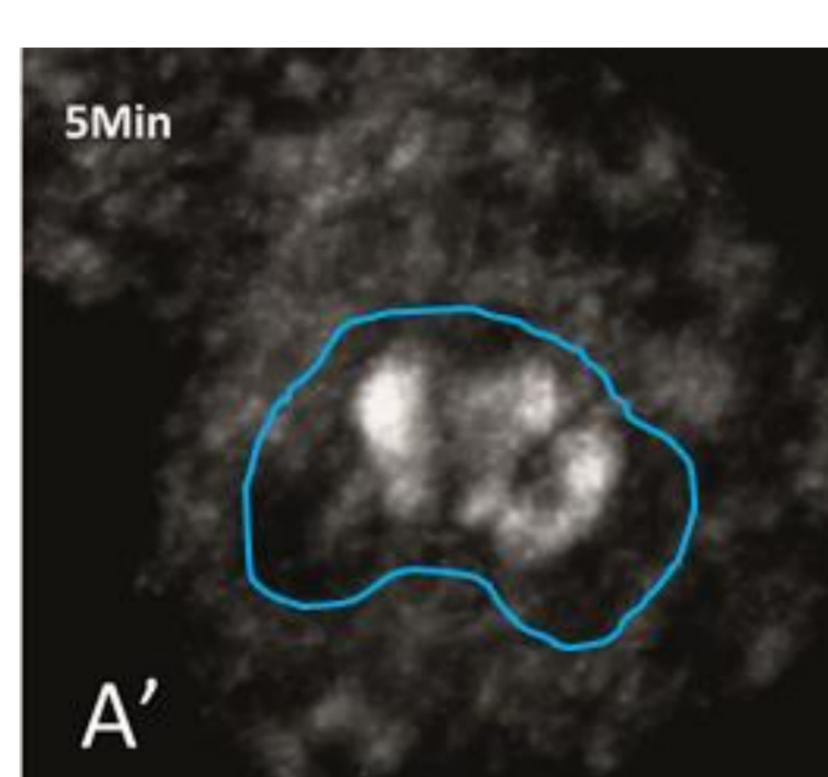
3A. The DNA is preferentially found in the fibrillar center (FC) in the tricompartmentalized nucleoli of lizard. Bar: 0,2 μ m (Lamaye *et al.*, 2011)



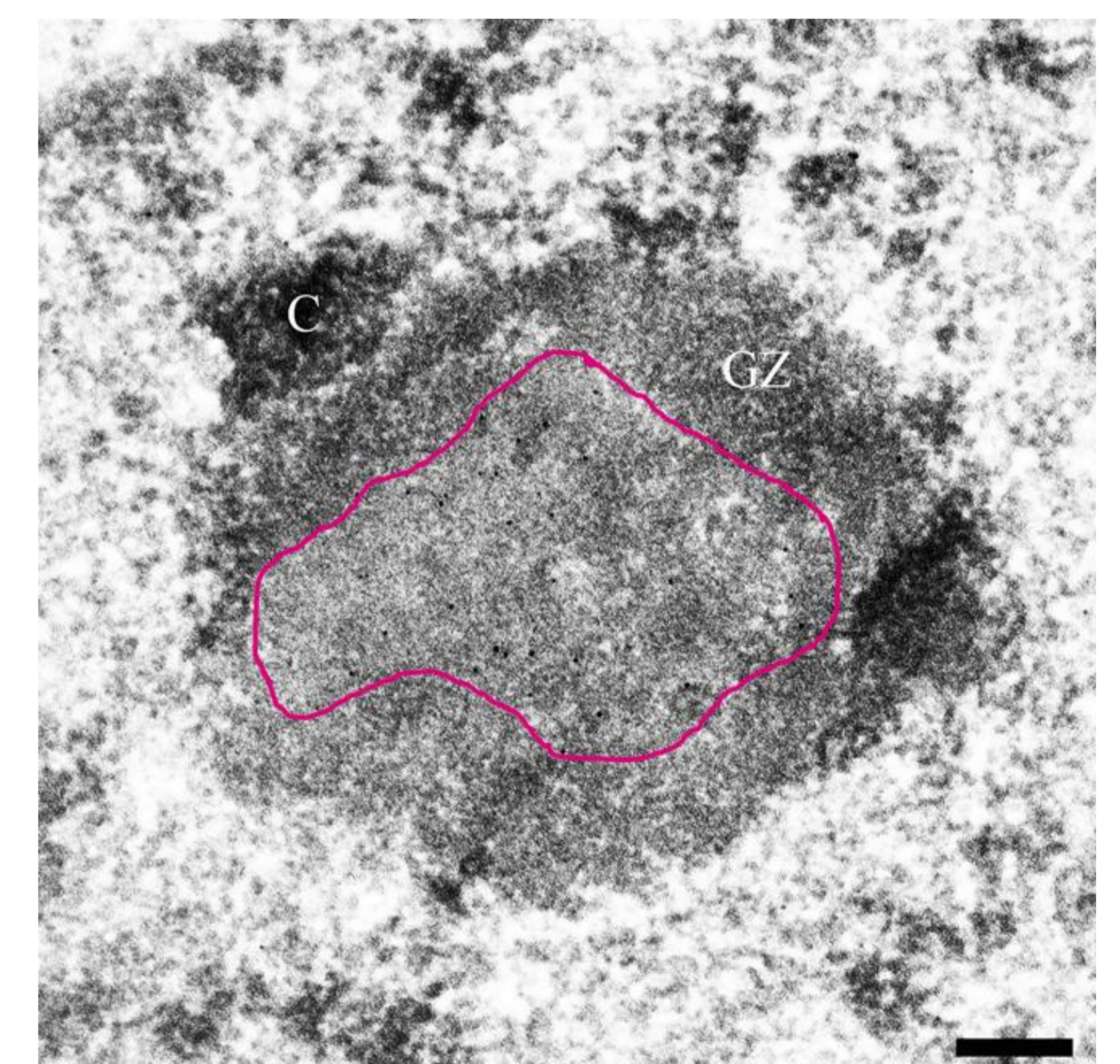
3B. The DNA is preferentially found over the dark strands (DS) of the fibrillar zone (FZ) in the bicompartimentalized nucleoli of turtle. Bar: 0,2 μ m (Lamaye *et al.*, 2011)



4. Dynamics of the immunolocalization of BrUTP-labeled rRNA in the tricompartmentalized nucleoli (Blue area) of lizard after a chase of 5 min (A) or 15 min (B).



4'. Homogenization of the immunolocalization of BrUTP-labeled rRNA in the bicompartimentalized nucleoli (Blue area) of turtle after a chase of 5 min (A') or 15 min (B').



5. Nascent RNA are preferentially found over the dark strands of the fibrillar zone (Pink area) in the bicompartimentalized nucleoli of turtle after 5 min of chase. Bar: 0,2 μ m

To localize DNA within each type of nucleolus, we used the terminal deoxynucleotidyl transferase immunogold method (Figure 3). For studying the spatial dynamics of RNA within the different nucleolar components, we used an uridine triphosphate analog, bromouridine triphosphate (BrUTP), which was transfected into cells then chased for different times before being detected by an immunocytochemical approach and then studied either by confocal microscopy (Figure 4) or by transmission electron microscopy (Figure 5).

Conclusions

Our results clearly indicate that in the lizard nucleolus the fibrillar center is the site of rRNA gene transcription, confirming previous observations realized on the tricompartmentalized nucleolus of mammalian cells. Furthermore, we reveal for the first time that the dense strands of the fibrillar zone correspond to the sites of rRNA synthesis within the bicompartimentalized nucleolus.

