First Evidence of Vasa Expression in Differentiating Male Germ Cells of a Reptile

The *vasa* gene has a central role in germ-cell development, and is conserved in animals. *Vasa* homologs usually exhibit germ-line-specific expression, so *vasa* mRNA and protein are generally utilized as germ-line markers. The gene encodes a member of the DEAD-box family of ATP-dependent RNA helicases that appears to regulate the translation of multiple mRNAs involved in germ-line differentiation (Gustafson and Wessel, 2010). During spermatogenesis, for example, the presence of Vasa was well documented in the cytoplasm of differentiating germ cells in many vertebrates and invertebrates (Gustafson and Wessel, 2010).

We used a species-specific anti-Vasa antibody to analyze, by confocal microscopy, Vasa expression during spermatogenesis in *Podarcis sicula*. The aim of the study was to assess if Vasa is involved in male germ line differentiation in this lizard, since no data are available to date on reptiles.

Vasa was specifically expressed in *P. sicula* male gametes from the early to the last stages of development, but was not detectable in mature spermatozoa residing in the lumen of the tubule (Fig. 1), as reported in other animals (Toyooka et al., 2000). In *P. sicula* spermatids, Vasa-stained spots aggregate into a single, large cytoplasmic body located in a notch of the nucleus (Fig. 1, inset). This structure may correspond to nuage material—the chromatoid body (CB) in spermatogenic cells—that is identifiable in the posterior part of developing spermatids in mice and rats. In these mammals, the CB is displaced toward the caudal pole of the nucleus of early elongating spermatids, where the bulk of the CB material condenses into a dense sphere, eventually forming a ring around the base of the developing flagellum (Shang et al., 2011). Because of its maintenance during male gamete development, the CB was proposed to have a role in spermatid cytodifferentiation. Indeed, as the egg germ plasm, the nuage-like CB includes proteins involved in the stabilization and translation of germ-cell-specific transcripts (Shang et al., 2011)—one of which is Vasa, considering that *vasa*-mutant mice exhibit a deficiency in post-meiotic progression before CB formation (Toyooka et al., 2000 and references therein).

In *P. sicula*, Vasa staining remains at one site of the spermatid elongated nucleus until the last stages of differentiation. At the periphery of the lumen of the *P. sicula* seminiferous tubule, for example, some germ cells similar to spermatozoa, but with a less-strongly packed chromatin, show intense Vasa staining at one extremity of the nucleus (Fig. 1); we believe these are spermatids in the last stages of differentiation, shortly before spermiation, the process during which sperm are released from the supporting somatic Sertoli cells into the lumen of the seminiferous tubule. The presence of Vasa until later stages of spermatid differentiation suggests that this protein may play an important role in the differentiation of *P. sicula* male germ cells.

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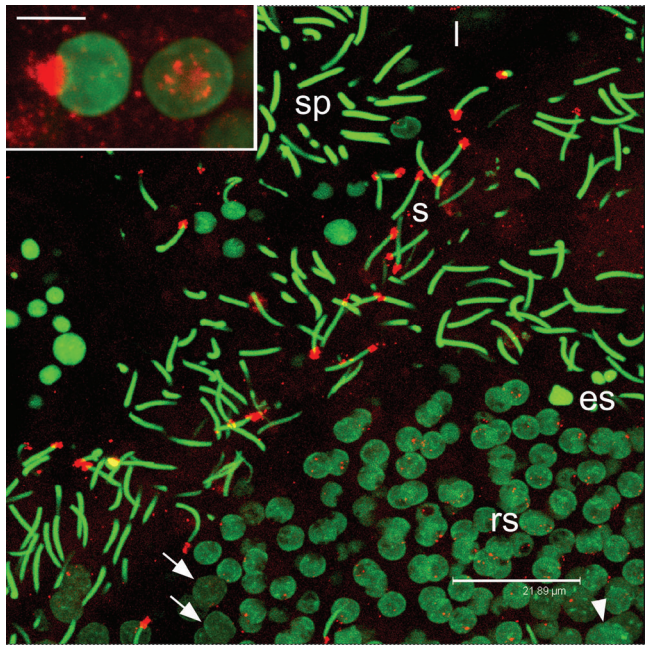

Figure 1. Testis cross-section of a *Podarcis sicula* adult during full gonadic activity. The image shows a portion of the apical region and the lumen of a seminiferous tubule. In the apical region, several stages of germ cells are visible: spermatocytes (I = arrowhead; II = arrows), round spermatids (rs), elongating spermatids (es). Spermatocytes and spermatids show cytoplasmic immunostained spots; in round spermatids, the spots converge in a notch of the nucleus (inset). In the peripheral region of the lumen, some germ cells, likely spermatids (s) in the last stages of differentiation shortly before spermiation, show strong Vasa staining at one extremity of their elongated nucleus. The tubule lumen appears full of spermatozoa (sp), some of which are sectioned transversely and others longitudinally. Spermatozoa are completely devoid of Vasa immunostaining. Anti-Vasa (in red) and TO-PRO 3 nuclear dye (in green). Scale bar, 21.89 µm; 5 µm in the inset.

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