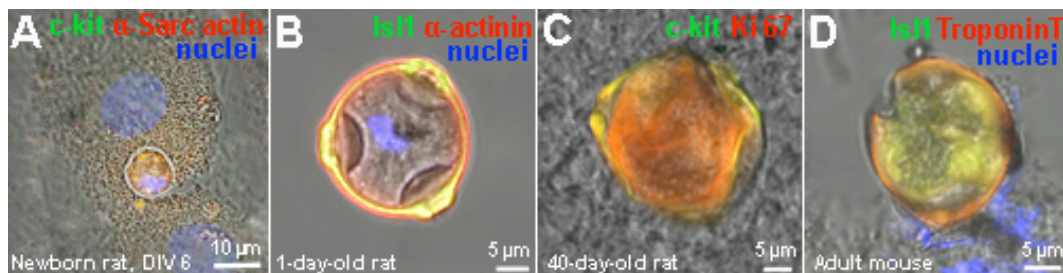


## Cell-in-cell development of resident cardiac stem cells inside mature cardiomyocytes: *in vitro* and *ex vivo* studies

«Cell-in-cell structures» (CICSSs) are usually defined as specific inclusions within the cells containing living cells of the same or distinct type. CICSSs may arise from such processes as phagocytosis, emperipolesis and entosis, and have been described in hematopoietic, immune, tumor and other cell types. In this work we first demonstrated the formation of CICSSs in the mammalian cardiac myocytes. Encapsulated CICSSs containing multiple small (5-7  $\mu\text{m}$  in diameter) cardiac stem cells (CSCs) belonging to c-kit<sup>+</sup>, Sca<sup>+</sup> and Isl1<sup>+</sup> subtypes were identified *in vitro* inside mature cardiac myocytes in the primary culture of myocardial cells of newborn, 20- and 40-day-old rats, as well as *ex vivo* in the suspension of freshly isolated myocardial cells of the rat, adult C57bl mice, cattle, and human (Fig. A-D). CSC-containing CICSSs were typically localized in the vicinity of the host cell nucleus (or between two nuclei) and covered by the membrane, separating CSCs from the cytoplasm of the host cell (Fig. A). Some CSCs confined to CICSSs expressed the marker of proliferation Ki67 (Fig. C). Repeated cycles of CSC proliferation and growth inside CICSSs resulted in the enlargement of CICSSs, which progressively occupied the intracellular space and dislocate the cytoskeletal myofilaments, nucleus, and other organelles. Expression of cardiac-specific proteins ( $\alpha$ -sarcomeric actin, sarcomeric  $\alpha$ -actinin and troponin T) indicates that the host cells are mature cardiac myocytes (Fig. A, B, D). It was shown that CSCs can undergo partial differentiation inside CICSSs, the latter resulting in the formation of cardiac transitory cells (TCs). It was also found that CICSSs development is terminated by the rupture of the capsule (Fig. D), followed by the release of cardiac marker-positive CSCs-derived TCs. On the basis of the data obtained in the primary culture of rat myocardial cells and *ex vivo* suspensions of cardiac cells of different mammal species it was supposed that lifelong self-renewal and regeneration of mammalian myocardium might, at least in part, occur by means of development of CICSSs-embedded CSCs inside the mature cardiomyocytes.

### Cell-in-cell structures formed by CSCs



Identification of cell-in-cell structures formed by resident CSCs inside cardiomyocytes in the culture (A) and in the freshly isolated myocardial suspension (ex vivo) of newborn rat (B), 40-day-old rat (C) and adult C57bl mouse (D).