EFFECT OF CLINOSTAT ROTATION ON LARVAL HEART DEVELOPMENT IN ILYANASSA OBSOLETA

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At some time during oogenesis or during the meiotic steps that are activated by and that then follow sperm entry, the morphogenetic determinants required for the differentiation of the larval heart become sequestered in the cytoplasm of the polar lobe that forms before and during first cleavage in fertilized eggs of the common marine mudsnail, Ilyanassa obsoleta Stimpson (= Nassarius obsoletus Say). If this cytoplasmic protuberance is removed at this stage, the larva that forms from the remaining two-cell embryo fails to form a heart and several other polar lobe-dependent tissues (J.W. Atkinson. 1971. J. Morphol. 133:339-352). Conversely, if the polar lobe is manipulated in such a way that it is cleaved in half during first cytokinesis, thus contributing equal amounts of polar lobe cytoplasm to each of the daughter cells of the 2-cell stage, several polar lobe-dependent tissues form in duplicate sets within the same embryo (J. Render. 1989. Devel. Biol. 132:241-250). We are interested in the steps by which cytoplasmic localization and distribution of cardiomyocyte differentiation determinants occur. In some cells, site-specific localization of cytoplasmic components, such as mRNAs or their translation products, require microtubules, cytoskeletal components that, in cells of many warm-, and cold-blooded vertebrates, oocytes of marine annelids and mollusks, and of protozoa, can be depolymerized by exposure to 0-4°C (L.G. Tilney and K.R. Porter. 1967. J. Cell Biol. 34:327-343; L.I. Rebhun et al. 1974. Nature 249:113-115; S. Inoué and H. Ritter, Jr. 1975. In: "Molecules and Cell Movement." (S. Inoué and R.E. Stephens, eds.) Raven Press, NY, pp. 3-30). When fertilized amphibian eggs are exposed to simulated microgravity by horizontal clinostat rotation, development is altered in a manner suggesting that the positions of the mitotic apparatus (or microtubule distributions) have been altered (H. Yokota et al. 1992. Int. J. Devel. Biol. 36:527-535; A.W. Neff et al. 1993. Devel. Biol. 155:270-274). In addition, patterns of microtubule polymerization have been shown to be affected by gravity vectors (J. Tabony and D. Job. 1992. Proc. Natl. Acad. Sci. USA. 89:6948-6952). In the experiments performed here, we reasoned that if the cytoplasmic determinants for heart development become localized to the polar lobe through the action of microtubules, then they might distribute abnormally within the fertilized egg if microtubule polymerization was compromised prior to first cleavage and/or if early development occurred until larval stages by continuous rotation of immobilized embryos on a clinostat. On the experimental clinostat, the axis of rotation was horizontal, i.e., perpendicular to the gravity vector (simulated microgravity), whereas on the control clinostat, the axis of rotation was vertical, i.e., parallel to the gravity vector (control). Capsules of freshly deposited fertilized Ilyanassa eggs (in which eggs are packed together in a viscous jelly) were collected immediately after their deposition by the female adult snail (insemination occurs before encapsulation) and either immobilized in tubes and placed on the control and experimental clinostats in matched sets, or first incubated on ice for 2 hrs (cold shock intended to favor microtubule depolymerization) before beginning control and experimental clinostat rotation. Rotation of control and experimental clinostats occurred at 7 rpm at room temp. (18-22°C) for 15-18 days. At the end of the incubation, embryos were released from capsules and immobilized physically so that their heart beat rates

could be determined. Results indicated that control and experimental embryos formed only 1 heart/larva. Although the hearts of adult Ilyanassa snails beat only slowly, those of larvae beat at 60-120 beats/min. Results from 10 experiments (8 capsules of control embryos vs. 8 capsules of experimental embryos in each experiment, with heartbeats determined in 6 embryos from each capsule) in which the eggs were first incubated at 0-4°C indicated no significant differences in the beat rates of control and experimental hearts (7), or faster in experimentals (1) or faster in controls (2). However, in those experiments (12) in which eggs were placed immediately on the clinostats (i.e., without any incubation on ice), in 6 experiments the heart beat rates were not significantly different, but in 6 experiments the experimental hearts beat at rates that were significantly faster than their matched controls. We conclude that there appear to be a set of non-iced incubation conditions that affect heart development, as assessed by larval heartbeat rates. Whether these differences from controls arise because of effects of simulated microgravity on microtubule distribution required for heart development remains to be demonstrated. Work supported by NASA-BioServe (NAGW-1197), NASA-NSCORT (NAGW-2328), and summer fellowship to A.P.S. from American Heart Assoc. (ME Affil.).