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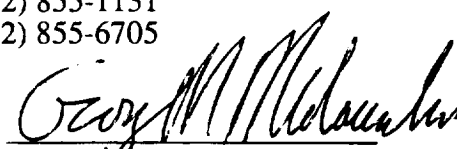
Technical Report for NAGW-1548

Gravity as a Probe for Understanding Pattern Specification

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Amphibian eggs from *Xenopus laevis* were employed as a model system. *Xenopus* embryos were demonstrated to be sensitive to novel force fields. Under clinostat-simulated weightlessness the location of the third cleavage furrow was shifted towards the equator (Fig. 1); the dorsal lip was shifted closer to the vegetal pole; and head and eye dimensions of hatching tadpoles were enlarged (Fig. 3). Effects of centrifuge-simulated hypergravity were the opposite of those of simulated weightlessness. Those morphological alterations had their own force-sensitive period, and a substantial spawning-to-spawning variation in sensitivity was observed. Despite those dramatic differences in embryogenesis, tadpoles at the feeding stage were largely indistinguishable from controls.

Embryo inversion and D₂O immersion were found to shift the third cleavage furrow location like clinostation, and cold shock (Fig. 4) was found to shift the furrow location like centrifugation (Fig. 5). Based on the additive or antagonistic effects of combined treatments, the primary cause of furrow relocation is postulated to be an alteration in the distribution of yolk platelets and the rearrangement of microtubule arrays. Centrifuged and cold-shocked embryos exhibited reduced survival in early developmental stages (Fig. 6). Cold-shocked embryos could be rescued by D₂O pretreatment or clinostating, an observation which supports the notion that changes accompanying furrow relocation represent the primary cause of the reduction in percent survival.

The morphogenesis of animal quartets (four animal blastomeres isolated at the eight-cell stage) differed depending on force treatments (Fig. 7). Animal quartets exposed to simulated weightlessness formed a groove and a protrusion more often than did controls or animal quartet exposed to simulated hypergravity. Molecular analyses revealed that transcripts of a dorsal lip-specific homeobox gene *goosecoid* and muscle-specific α -cardiac actin were detectable in the animal quartet with the projection (see Fig. 7). All results were consistent with the notion that the shifted partitioning of morphogenetic information in animal quartets is a primary cause of force-dependent differential morphogenesis and gene regulation.

These data provide an important foundation for interpreting actual spaceflight results for embryos flown on various missions.

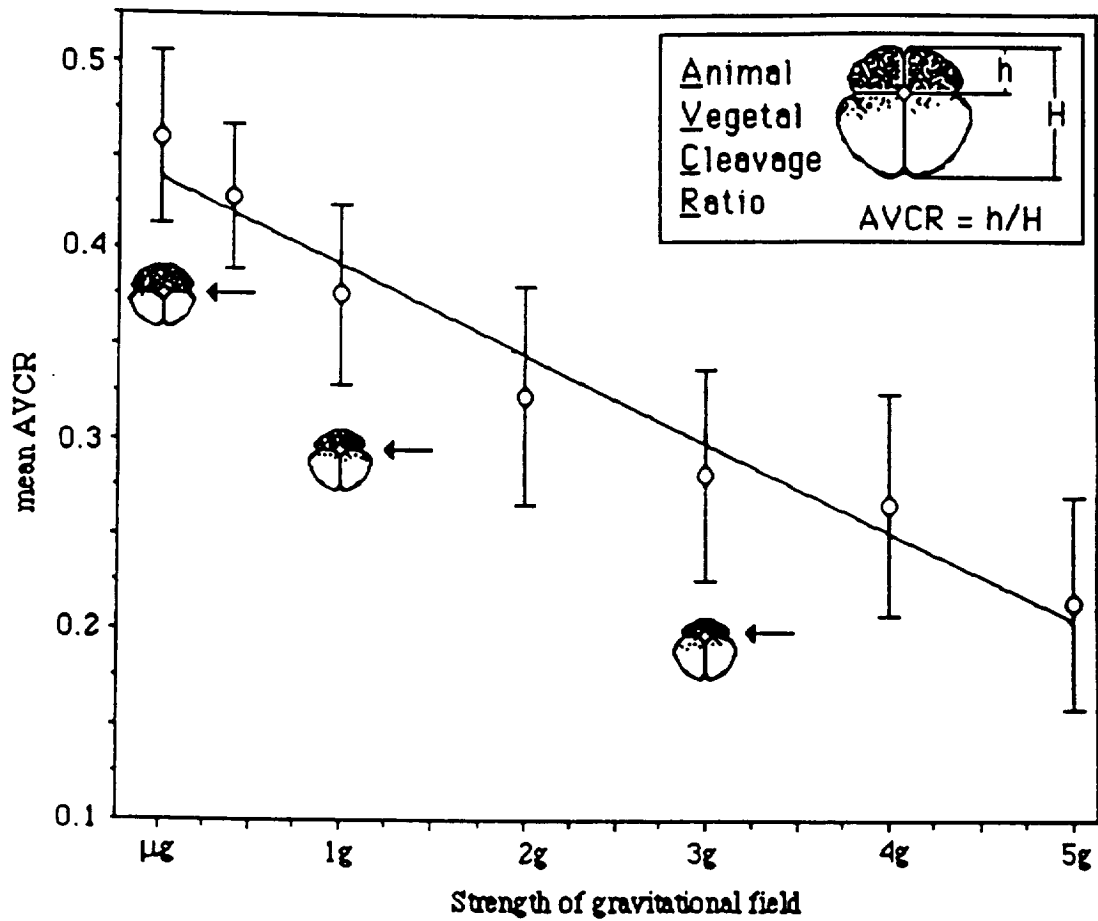


Fig. 1. The definition of AVCR (inset) and the relationship between mean AVCR and the strength of the gravitational field. The mean AVCR decreases in a linear fashion as the strength of the gravitational field increases. A linear best fit line is expressed as $(\text{mean AVCR}) = 0.433 - 0.08 X$ (the strength of gravitational field) with $r = 0.98$. Each data point represents the mean AVCR value from 4 to 28 spawnings. A total of 1233 embryos were analyzed. One standard deviation is shown. Arrows point to third cleavage furrow.

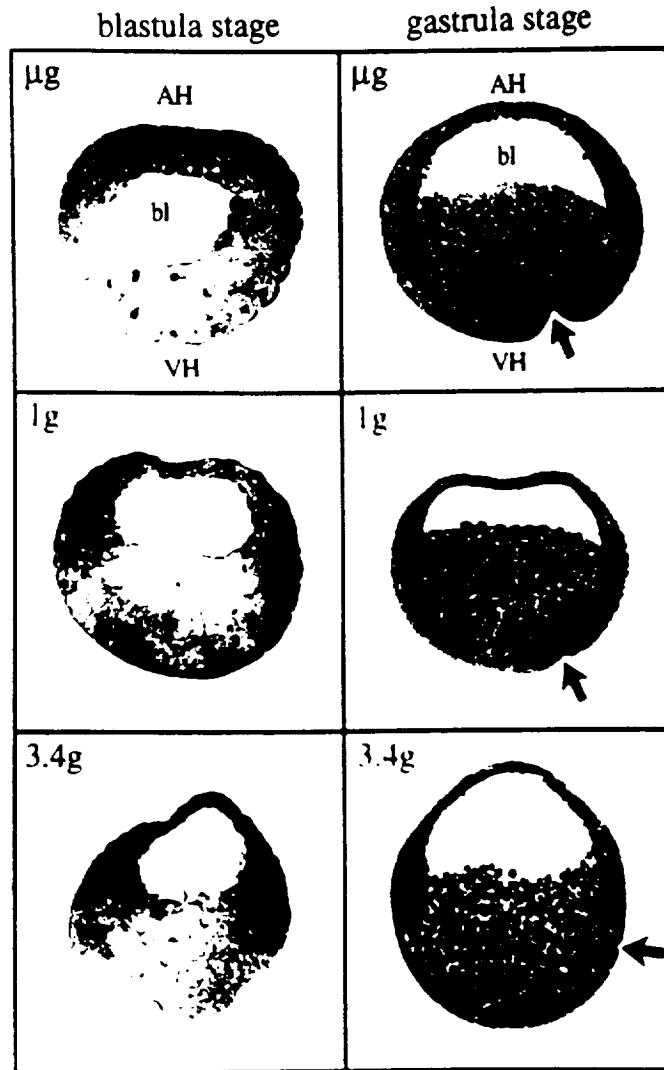


Fig. 2. Methacrylate-embedded embryos exposed to " μg ", "1g" (control) and "3.4g". Left: Cross sections of midblastulae (stage 8) showing the dramatic gravity effect on the location of the blastocoel and on the thickness and the number of cell layers in the blastocoel roof. Right: Midsagittal sections of early gastrulae (stage 10) showing the altered location of involution site (arrows). The location of the blastocoel, and the thickness and number of cell layers in the blastocoel roof are regulated toward normal. Abbreviations: bl-blastocoel; AH-animal hemisphere; VH-vegetal hemisphere.

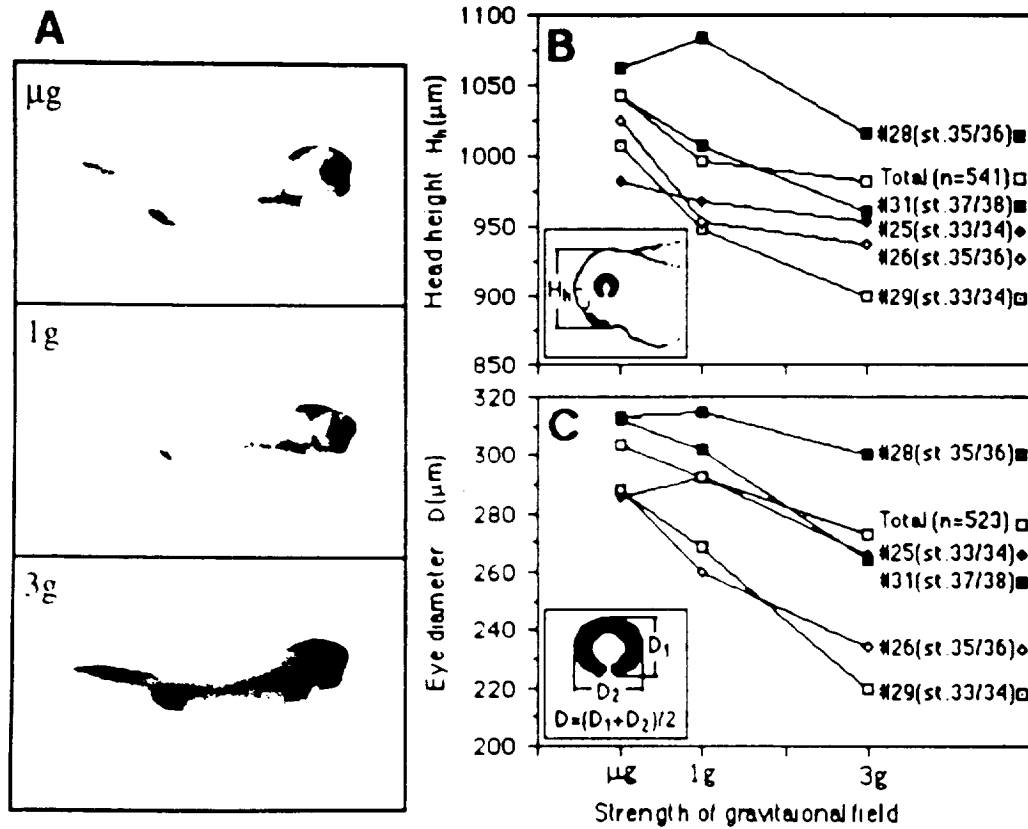


Fig 3. Subtle dimorphogenesis along the anterior/posterior axis of heart beat/hatching stage embryos by gravity simulations. A. Hatched larvae (state 36/37); B. Comparison of head height; and C. Comparison of eye diameter. μg -treated larvae show a slightly exaggerated dorsal curvature and anteriorization exemplified by increased head height, eye diameter and anterior thorax height. Despite spawning-to-spawning variation (five spawnings: #25, #26, #28, #29 and #31), there is a significant difference in both total mean head height and total mean eye diameter between μg -exposed tadpoles and 1g-controls, and 3g-exposed tadpoles and 1g-controls (t-Test at $p < 0.05$).

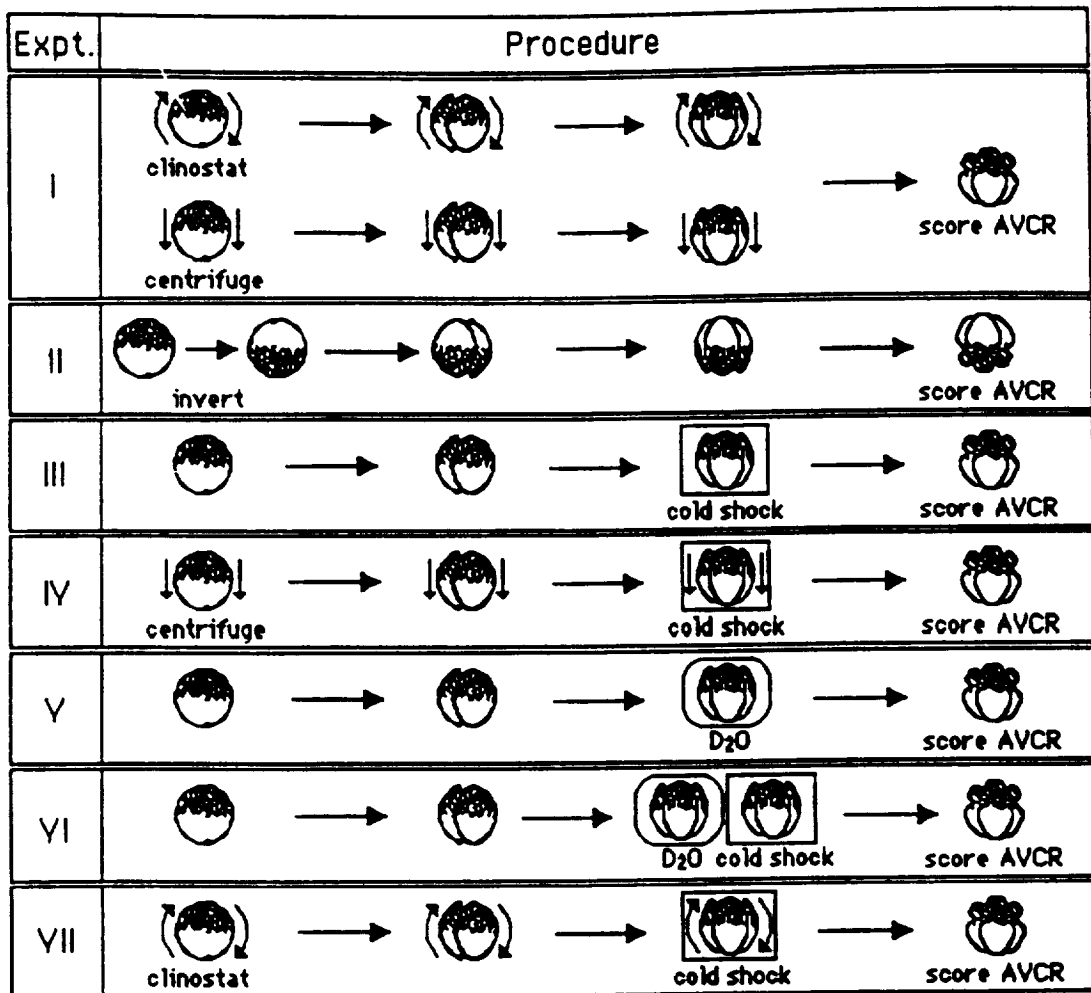


Fig. 4. Summary of experimental designs in Chapter 3. AVCR was scored at the eight-cell stage after exposure to a variety of treatments such as the clinostat or centrifuge (Expt. I), embryo inversion (Expt. II), cold shock (Expt. III), centrifuge and cold shock (Expt. IV), D₂O immersion (Expt. V), D₂O immersion and cold shock (Expt. VI), and clinostat and cold shock (Expt. VII).

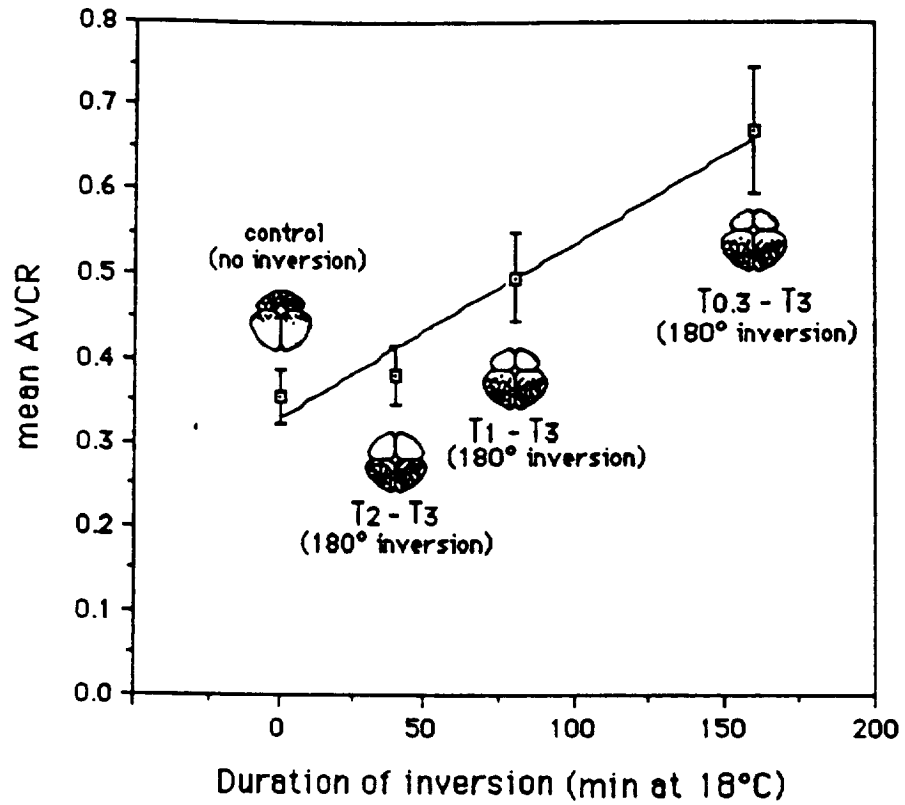


Fig. 5. A linear relationship between AVCR and duration of inversion. Based on the pooled data (5 spawnings, 282 embryos), the best fit line is expressed as “(AVCR estimate) = 0.0021 X (duration of inversion in minutes) + 0.33” with $r = .098$. One standard deviation is shown.

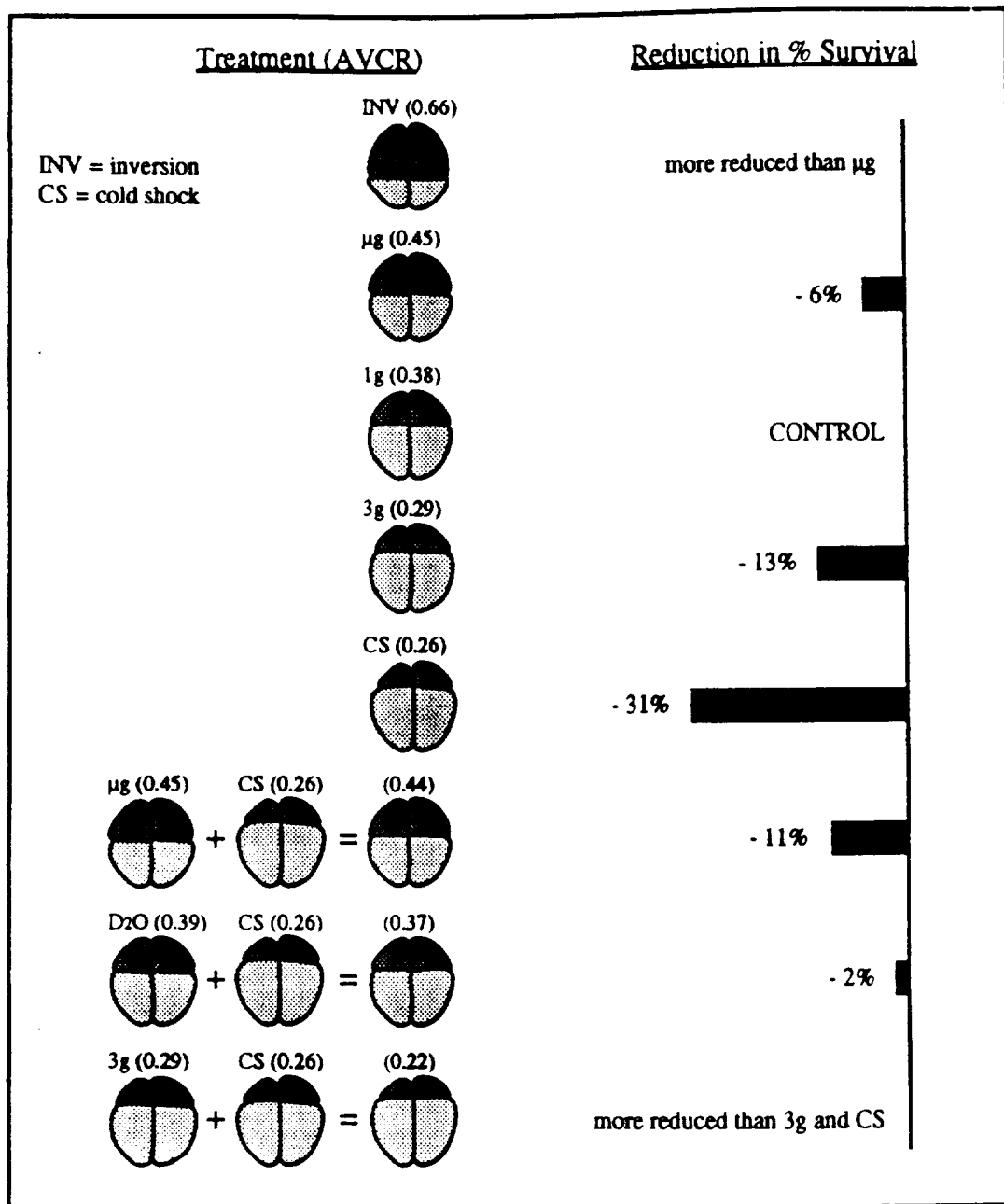


Fig 6. Schematic summary of the cleavage ratio analysis. Mean AVCR values and the reduction in percent survival are summarized with various treatments such as egg inversion, μg -exposure, 3g-exposure, cold shock, and a combination of cold shock with μg -, D₂O or 3g-treatment.

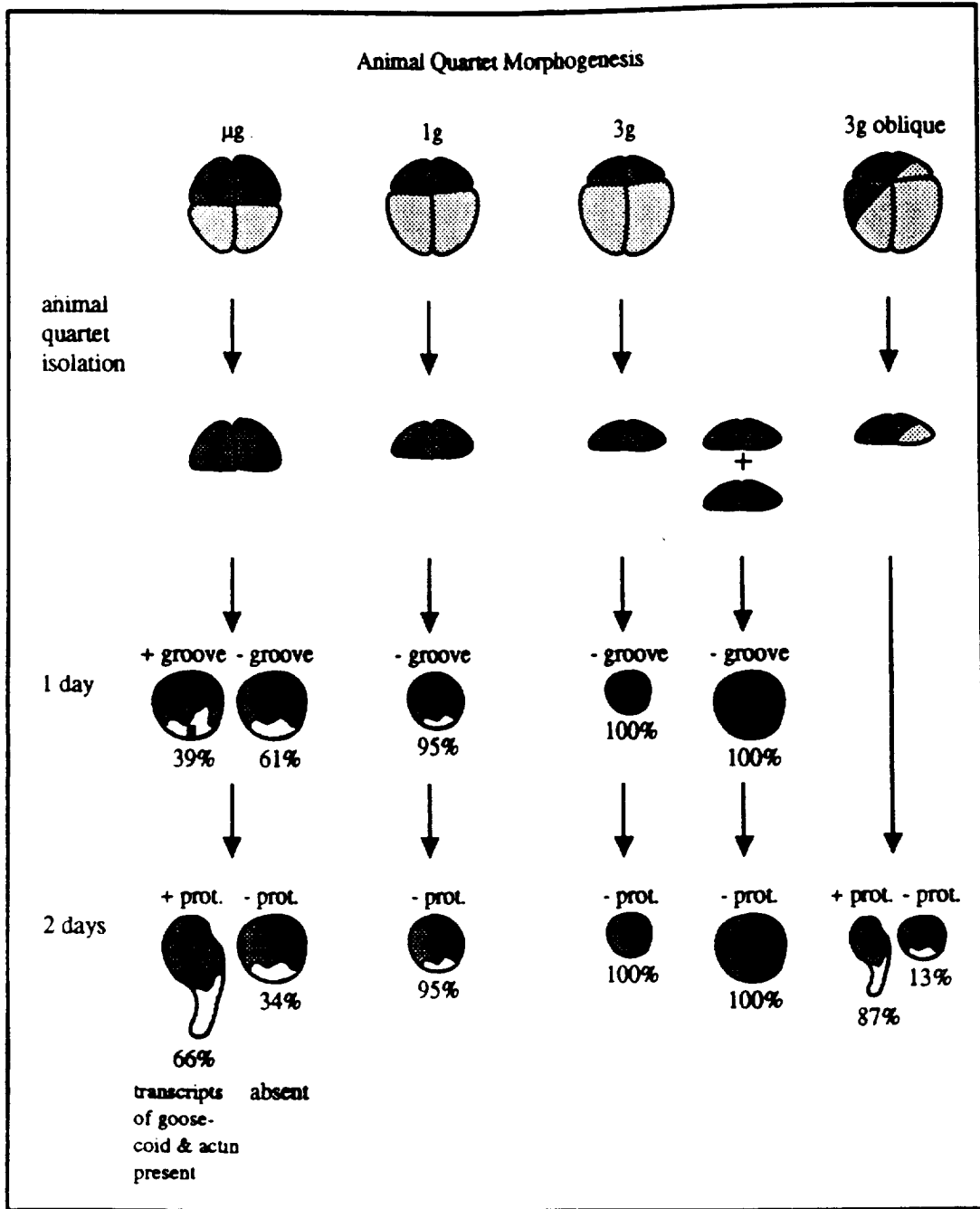


Fig. 7. Schematic summary of the animal quartet analysis. At the eight-cell stage, intact animal quartets consisting four animal blastomeres were isolated, and their morphogenesis was examined one day and two days after isolation. Transcripts of the dorsal lip-specific homeobox gene, goosecoid, and α -cardiac actin were detectable by PCR in the animal quartets with a protrusion (abbreviation: prot. = protrusion).