EXPERIMENTAL ANALYSIS OF THE MECHANISMS IMPLICATED IN THE INDUCTION AND COMMITMENT OF PRECARDIOGENIC MESODERMAL CELLS DURING AVIAN GASTRULATION.

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In avian embryos at the gastrula stage, prospective mesodermal cells located in the cranial primitive streak are ingressing and moving from medial to lateral to form either a paired precardiogenic mesodermal area (early gastrula) or somitic paraxial mesodermal area (late gastrula; Schoenwolf et al., 1992; Garcia-Martinez and Schoenwolf, 1993). Thus, cells from the cranial primitive streak at different stages have different fates and patterns of displacement during development. The factors controlling cell movement and fate during gastrulation and the timing and mechanisms of induction and differentiation of the mesodermal cells are poorly understood. In the present study we analyze the results of experiments designed to elucidate mechanisms underlying the establishment of the pattern and differentiation of precardiogenic mesodermal cells. We graft heterotopically and heterochronically groups of precardiogenic quail cells into chick host embryos, subsequently analyzing the distribution and gene expression of these cells using immunocyochemistry and *in situ* hybridization.

Materials and Methods

Experimental procedures

Fertile White Leghorn chick and Japanese quail eggs were incubated to stages 3 to 5 (Hamburger and Hamilton, 1951, with substaging 3a-3d according to the criteria of Schoenwolf et al., 1992). Chick embryos as hosts and quail embryos as donors were cultured ventral-side up on their vitelline membranes according to New (1955). Four experiments were performed (Figure 1), all with heterotopic grafts. In type-1 experiments, an area of the donor (stage 3a-b) primitive-streak 250-375 μm caudal to the rostral end of Hensen´s node, designated in previous studies (Schoenwolf et al., 1992; Garcia-Martinez and Schoenwolf, 1993) as site C1 was grafted to site C1 of host primitive-streak (stage 3d-4). Based on our homotopic, isochronic fate-mapping studies, site C1 at stage 3a-b contributes cells to endocardial and myocardial layers of primitive cardiac tube, whereas site C1 at stage 3d-4, contributes bilaterally to cranial somites. Type-2 experiments were the reciprocal of type-1. In type-3 experiments, a segment of the precardiogenic area at stage 5 (lateral to the head process), was removed from the donor and placed isochronically in a more caudal, non-precardiogenic area in the host. In type-4 experiments, Hensen´s node from donors at stage 3d-4 was placed isochronically to the germ cell crescent of the host.

Graft-specific label and tissue- and cell-specific markers

Grafted cells were identified with anti-quail antibody and visualized using peroxidase immunocytochemistry. In addition, tissue- or cell-specific markers were used to analyze and identify grafted cells within an organ rudiment at the end of each culture period. The markers used were: a riboprobe transcribed from the chick paraxis cDNA (D. Sosic and E. Olson), which is expressed in the somites and rostral segmental plate mesoderm; and the cardiac muscle marker cNkx-2.5, a chick homeobox-containing gene that shares extensive sequence similarity with the *Drosophila* gene tinman (Schultheiss et al., 1995). Both were visualized using whole mount *in situ* hybridization. The use of anti-quail after *in situ* hybridization allowed the visualization of cells double labeled in whole mount and histological sections.

Results and Conclusions

Our results show that primitive streak precardiogenic cells, placed into primitive streak at the location and stage characteristic of the presomitic mesodermal cells (type-1 experiments) are able to acquire somitic phenotype. As can be seen in Figure 2, quail cells have formed somites and may express paraxis (this paraxis riboprobe labels quail less strongly than chick in control embryos). In the reciprocal experiment (type-2), presomitic cells from the primitive streak transferred to the precardiogenic region of the primitive streak in younger embryos contribute cells to the heart and express the cardiac marker tinman (Figure 3). Type-3 experiments show that mesodermal cells from a lateral precardiogenic area maintain their expression of tinman even when they are placed in a noncardiogenic region (Figure 4). These data suggest that precardiogenic cells are labile and able to change their fate and their gene expression pattern at the time of their ingression through the primitive-streak, but not afterward when they have formed the mesodermal layer. Finally, type-4 experiments show

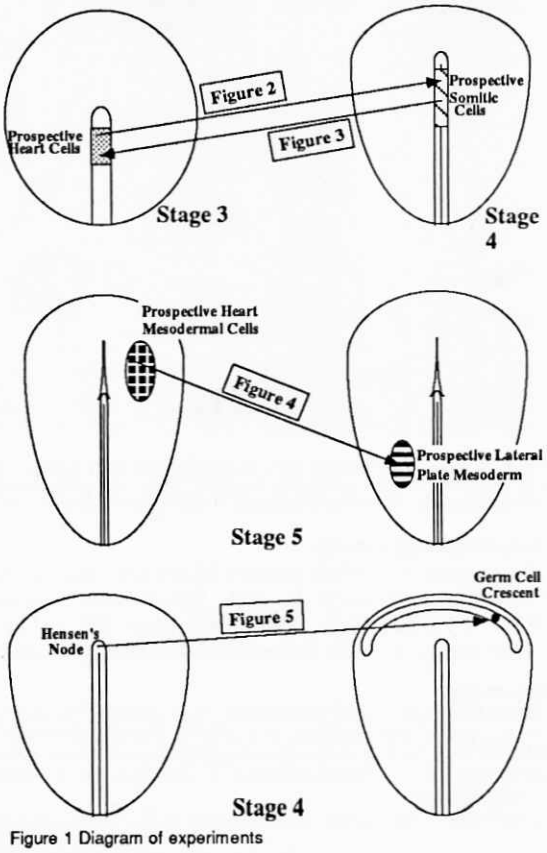

Figure 1 Diagram of experiments

that ectopic hearts can be organized within the extraembryonic region of the blastoderm and can express the cardiac marker tinman after heterotopic grafting of Hensen's node. As can be seen in Figure 5, quail cells from the donor Hensen's node in the germ cell crescent lie adjacent to the neighboring host cells that express tinman. The precise role of Hensen's node in the formation of the ectopic heart cannot be ascertained from this study, and additional experiments are underway.

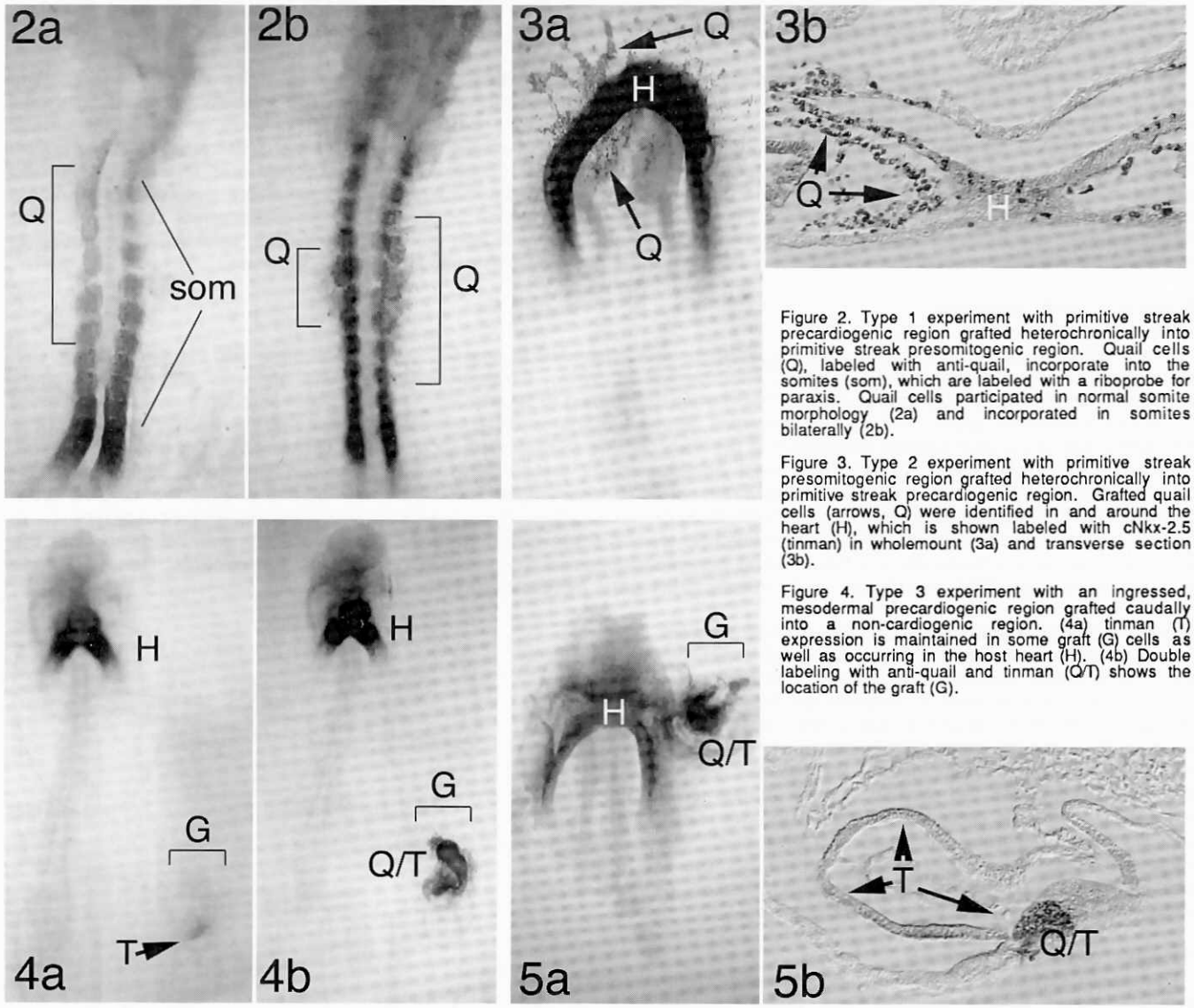

Figure 2. Type 1 experiment with primitive streak precardiogenic region grafted heterochronically into primitive streak presomitogenic region. Quail cells (Q), labeled with anti-quail, incorporate into the somites (som), which are labeled with a riboprobe for paraxis. Quail cells participated in normal somite morphology (2a) and incorporated in somites bilaterally (2b).

Figure 3. Type 2 experiment with primitive streak presomitogenic region grafted heterochronically into primitive streak precardiogenic region. Grafted quail cells (arrows, Q) were identified in and around the heart (H), which is shown labeled with cNkx-2.5 (tinman) in wholemount (3a) and transverse section (3b).

Figure 4. Type 3 experiment with an ingressed, mesodermal precardiogenic region grafted caudally into a non-cardiogenic region. (4a) tinman (T) expression is maintained in some graft (G) cells as well as occurring in the host heart (H). (4b) Double labeling with anti-quail and tinman (Q/T) shows the location of the graft (G).

Figure 5. Type 4 experiment with Hensen's node from quail grafted into the germ cell crescent and cultured shows the host and graft (G) in whole mount (5a) and part of the region containing the graft (G) in transverse section (5b). Embryos were labeled with anti-quail and tinman. tinman (T) labeled the heart of the host embryo (H) as well as the tissue in the region in and around the quail graft (Q).

Acknowledgments

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