Beyond the Heart of the Matter: Other Effects of the Cardiac Mutation

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As originally described by Humphrey (1972), the *c* gene prevents the onset of heartbeat. Other defects are also found in *c/c* larvae: they are edemic and microcephalic, have reduced gills and stature, abnormal pigmentation, and aphagia—the inability to feed. These defects were believed to be caused by the absence of circulation. If the effect of *c* were only the heart, the presence of a functional circulatory system would be sufficient to rescue mutant embryos.

As noted in the preceding paper (Armstrong and Smith 1993), we have been examining the effects of transplanting wild-type heart mesoderm into *c/c* embryos. In many cases we were able to obtain mutant embryos with beating hearts and circulation. At first, we were elated by the prospect of raising, and eventually spawning, these animals. Visions of 100% mutant spawnings danced in our heads. Imagine not spending 3/4 of our time operating on wild-type sibs (or waiting until they reached stage 35), and never again having to rely on nasty statistical analyses or explant cultures to interpret our results.

It was a beautiful vision. Unfortunately, it wasn't meant to be. Much to our chagrin, when we managed to get embryos with beating hearts and circulation, we discovered that *c* appears to affect at least two tissues other than the myocardium. Although usually masked by the failure of the circulatory system, these defects became apparent in our "rescued" mutant larvae.

Bilateral transplantations. When the heart primordia in stage 20 *c/c* were bilaterally replaced with wild-type heart mesoderm, beating hearts formed in 6/7 cases (Table 1 in Armstrong and Smith 1993). Circulation began in all of these embryos, and in most wild-type recipients of wild-type tissue.

In such embryos, most of the defects associated with the cardiac mutation were absent: they were not edemic, had normal heads, gills, and pigmentation, and were the same size as their wild-type sibs. However, the "rescued" larvae remained aphagic, and slowly starved to death—hearts beating, and circulation continuing until the end.

Some hosts had defects resulting from incomplete healing, usually misshapen opercula, missing primary gill filaments, and slightly extruded organs (usually the liver). These larvae were often unable to feed. But wild-type larvae without such obvious external defects ate and grew normally. Of 6 *c/c* recipients of bilateral transplants, 3 had surgical defects. Of the remainder, 2 were completely aphagic. They made normal feeding strikes, but were unable to swallow. The remaining "rescued" larva swallowed a little food, but much less than its wild-type counterparts. This larva was from an abnormal spawning, however. Unoperated mutant larvae from this spawning (# 224-9 X 221-1) were also able to swallow small amounts of food.

The aphagia of these larvae did not appear to be due to surgical damage. Wild-type sibling hosts without surgical damage were able to feed, but similarly undamaged *c/c* hosts could not. Therefore, the aphagia of cardiac mutant larvae appears to be due to a separate effect of the *c* gene. The spawning where control *c/c* larvae could swallow some food supports this conclusion, since it indicates the aphagia in *cardiac* larvae is unrelated to their lack of circulation. It may also indicate that *c* has changed, at least in one strain of animals in the Ottawa colony.

We are uncertain what causes the inability to feed. Since mesoderm is affected by *c* (Smith and Armstrong 1991a, b, Armstrong and Smith 1993), one possibility is that the mandibular mesoderm (found at the anteriormost edge of the mesodermal mantle, next to the heart mesoderm) is affected. This tissue was not replaced during our transplantations.

Unilateral transplantations. As well as replacing heart mesoderm bilaterally in stage 20 *c/c* embryos, in another series we replaced only one of the paired primordia. While testing whether the presence of a single primordium could stimulate the formation of a functional heart (Armstrong and Smith 1993), we also hoped to minimize the surgical trauma to the

hosts (and maybe get some that could swallow!). Beating hearts formed in almost all embryos containing at least one wild-type heart primordium, as shown in the preceding paper (Table 2). Circulation began in many of these embryos, but less frequently in those with chimeric hearts (2/6 *c/c*, and 8/13 +/+ hosts) than in mutant hosts with bilateral transplants of wild-type tissue (6/6 mutant hosts).

More interestingly, the circulation stopped within a few days in all embryos with chimeric hearts. The hearts continued to beat vigorously, but circulation did not resume. The larvae became severely edemic, and began to resemble control *c/c* larvae. Their blood pooled in several capillary beds (particularly the gills, pronephroi, and liver), in the coronary arteries, and in the heart itself. Circulatory arrest was never observed in larvae containing only wild-type tissues, indicating that it is unlikely to have been caused by surgical damage.

This suggests that there is a morphological defect in hearts which contain *c/c* tissue which cannot be corrected by stimulating myofibrillogenesis in the myocardium. The nature of the defect remains unclear, but our observations suggest one possibility: the circulatory arrest may be indicative of a heart valve defect.

In axolotl embryos, heartbeat is initially quite weak and peristaltic. Only later (stage 38-39) does the alternating beat characteristic of mature hearts begin. During early stages, the sweeping contractions of the heart may be sufficient to keep the blood flowing, but later valves would become necessary to prevent blood from flowing back and forth between the heart chambers. Blood moving in this manner was seen in several embryos with circulatory arrest. The resulting loss of arterial pressure could also cause blood to pool in the capillaries. Lemanski and Fitzharris' (1989) report that the number of endocardial and mesenchymal (cardiac cushion) cells is greatly reduced in the anterior regions of mutant hearts supports this hypothesis. Cardiac cushion mesenchyme forms the heart valves (see Patten et al. 1948, Manasek et al. 1984).

Conclusions. We have uncovered evidence for two other effects of the *c* gene. The aphagia observed in *c/c* larvae cannot be caused by the lack of circulation, as previously suspected (Humphrey 1972), but may be directly caused by *c*. There also appears to be a defect in the heart valves, which is not corrected by stimulating *c/c* myocardium to begin beating. These results suggest that the *c* gene has broader effects than originally believed. Furthermore, our observation of mutant larvae which can swallow suggests that the cardiac gene may be changing in at least one strain in the Ottawa colony.

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