THE EYELESS MUTANT AXOLOTL: SOME CONTRADICTIONS AND SOME MORE NEGLECTS.

Rudolf B. Brun

Texas Christian University

Biology Department

Fort Worth, Texas 76129

Last summer Cuny and Malacinski published a paper including data on the eyeless mutant axolotl. (JEEM 96, p.151, 1986). A major part of these results were obtained by culturing prospective eye tissues from both wild type and eyeless mutant embryos. Cultures were prepared by explanting anterior neural plate (the prospective region of the optic cup tissues). Neural plate material was combined with epidermis to form "sandwich cultures". After culturing, the explants were analyzed for the presence or absence of neural retina, pigmented retina, and lens. Cuny and Malacinski ('86) found that neural plate from eyeless mutant embryos formed less eye structures *in vitro* as compared to anterior neural plate from wild type embryos. They concluded that the presumptive retina cells were the primary target of the mutant gene *e* .

This conclusion contradicts previous results obtained with the eyeless mutant (Brun, American Zool. *18* ,p. 273, 1978). Grafting of the prospective optic cup from the mutant under flank epidermis of normal hosts revealed that the eye region of the mutant was capable of forming eye tissues including normal eyes. Pigmented retina from mutant tissue was observed in 12 grafts out of 14 (86%) and three normal eyes formed (21%). In controls, the grafting of 53 wild type prospective optic cups under flank epidermis of normal hosts led to 50 cases of

ectopic pigmented retina formation (94%) and 18 normal eyes (34%).

These results suggested to me that the optic region of the mutant might be normal but prevented from differentiating in situ by neighboring cells or tissues. One possibility was that neural crest cells might be involved in suppressing the eyes from forming (see Ulshaver and Hibbard, Anatomy and Embryology 156 1979). This hypothesis was tested and the results were recently published (Brun, Journal of Neurogenetics 4 , p.29, 1987). An alternative hypothesis, namely that the epidermis of the eyeless mutant might be involved in causing the mutant phenotype was also investigated (Brun, '78) with the following results: Replacing the mutant epidermis in the eye region with flank epidermis from wild type embryos, led to the formation of mutant pigmented retina in situ in four out of 12 cases (33%). Normal eyes differentiated three times out of these twelve grafts (25%). Both types of grafting experiments demonstrated that the prospective optic cup region of the mutant was capable of forming eyes. Van Deusen (Dev. Biol. 34 ,p.135 1973) had already demonstrated that cells from the prospective eye region of the mutant were capable of participating in pigmented retina formation (Van Deusen's Table 3).

From this background, I have difficulties to understand why Cuny and Malacinski ('86) concluded that the defect caused by gene e is intrinsic to the neural plate of the mutant. It was quite surprising to me that these authors did not discuss or

mention Van Deusen's findings (the results listed in his Table 3) or the results I published in 1978:

Why?

I hesitate to accept the main results published by Cuny and Malacinski ('86) for the following reasons: As I understand their paper, the main method used for analyzing the eye forming tissues of normal embryos and mutant neurulae was to explant neural plate together with epidermis in vitro . In such cultures prepared from normal embryos selected from parents heterozygous for gene e , 100% of the cultures produced neural retina and 95% pigmented retina (Cuny and Malacinski '86, p.161, Fig. 5, line 1). However, in similar cultures also prepared from wild type neurulae, none of the cultures produced any eye structures (Fig. 5, line 2). This discrepancy in eye tissue formation between normal embryos selected from heterozygous parents, and wild type neurulae from wild type parents does not make sense. Is there a printing error or do I miss the point for other reasons? Perhaps the data in the two lines should be combined? If that is the case, 36 cultures were prepared from wild type neurulae: 22 (61%) formed neural retina, pigmented retina was found in 21 (58%) of these cultures while lens tissue was observed in 10 (24%) of the cultured explants. In other words, 39% of the control cultures prepared from wild type donors did not produce any eye tissues.

The cultures for the experiments listed in Fig.9, were produced using neurulae obtained from parents heterozygous for

gene e . In such spawnings only 25% of the embryos are homozygous for the mutant gene. How is it possible to identify cultures produced from tissues of these 25% of the embryos, if wild type controls show the "mutant phenotype" at a rate of 39%? The authors must have been aware of this problem because there was an attempt to overcome the difficulty in the following way: At the time the neural folds just start rising, one half of the anterior neural plate was excised from one side of the neurula and used for the cultures. The contralateral side was left in the embryo to see whether or not it would develop an eye. If the larva had an eye on its right side, the cultured piece of the neural plate was classified as wild type. If no eye devloped the neural plate excised from the left side was classified as being of the eyeless mutant genotype . I doubt that it is possible to excise one side of the eye primordium at this stage without at least occasionally creating the "eyeless phenotype" in genetically wild type larvae. There is one sentence in their paper addressing this rather crucial problem (p. 153: Organ culture). Cuny and Malacinski warned the reader from making "jagged cuts" because they led to false results. A control, however, dealing with the problem of how many times the anterior neural plate excision might produce "false eyeless", is missing. Also missing is an explanation, why there is oscillation in eye tissue formation in the controls between 100% (Fig.5, line 1) to 0% (Fig. 5, line 2), but not in the experiments with eyeless mutant tissues presented in Fig. 9.

In summary: The main experimental data in Cuny and Malacinski's paper are debatable in my opinion, because of the oscillating results produced by their method of culturing the explants. Of course I cannot exclude that I do not fully understand their data. I invite the authors to provide additional enlightenment in this issue of the Axolotl News Letter, so that I can adequately deal with this matter in future grant proposals and papers. I seriously hope that the authors will also use this opportunity to offer an explanation, why results contradicting their main conclusion were not discussed or even mentioned in their paper.

It seems crucial to me that senior researchers feel responsible for junior investigators including the case, were they are "only" coauthors. Carelessness in this matter can cause serious damage. For example reviewers of grant proposals occasionally seem to depend too heavily on the information (or lack of it) in the discussion part of the most recent paper which happens to be published in the field.

The fact that the reviewers of a prestigious journal like JEEM failed to correct this damaging neglect, is an additional, still more concerning matter.