

Note from the Director

We continue with our attempts to develop protocols for generating transgenic axolotls. Our strategy is to focus on each of the component steps which might comprise an ultimately successful procedure. Using the methods employed with *Xenopus* as a guide we have accomplished several of the steps. (See: <http://www.welc.cam.ac.uk/~ea3/The.Amaya.Lab.Homepage.html>)

First, sperm are collected by dissection of freshly collected vas deferens, and concentrated to an appropriate density (so that during subsequent microinjection each egg will receive 1-5 sperm). Second, sperm heads are swollen, so that when bathed in a solution of a foreign gene a suitable amount of gene will enter each sperm. This "swelling of sperm" is accomplished by adding 2.0-5.0 M NaCl to a sperm suspension. Within two hours at room temperature the sperm heads swell to several times their natural volume. Third, those swollen

sperm are injected directly into freshly spawned, dejellied axolotl eggs, in order to determine whether they can drive cleavage. It is here that we are presently "stalled." Cleavage frequency varies dramatically from spawning to spawning.

Only approximately 10% of the injected eggs from some spawns cleave while from other spawns up to 25% cleave. Of those which cleave normally, only a fraction (approx. 25%) gastrulate, and only a few of those neurulate. We will work at treating the sperm in a less harsh fashion in an attempt to improve development of recipient eggs.

Once cleavage is achieved with a reliable frequency we plan to treat the swollen sperm with an appropriate foreign gene. Dr. Roy Tassava of Ohio State University is working closely with us on all aspects of this project and will provide a gene construct.

George M. Malacinski

News from the Axolotl Colony

Update on Fees

The Axolotl Colony has been charging for material for a bit over a year. We revised the fee schedule for the current season in order to make it fairer and easier to administer, while still trying to keep paperwork fairly simple. Prices for embryos are somewhat higher than last season, and there is no longer a cost ceiling for high volume embryo users. These charges help, of course, to defray some of the costs we incur in order to supply the material, but at this time they do not nearly cover all of the expenses involved in running the colony. The new price structure and invoicing system for embryos also reflects how different laboratories use embryos. Our embryo users fall mainly into two groups, those that use fewer than 1500 embryos and receive fewer than 10 shipments, and those that use greater than 3500 embryos and receive 20 or more shipments over the course of the season. The former will now be invoiced monthly, while high-volume users with standing orders will be invoiced quarterly. We hope this system will keep paperwork manageable.

We would like to thank all of you who have

used our services during this period of adjustment for your patience and understanding as we learn and improve.

Also, please note: All users of Axolotl Colony material are required to register with the colony (one registration per lab), but you do not need to register more than once. Please do, however, let us know if your address, telephone, e-mail, etc. change. If your institution requires a purchase order, please have the number available when you make your order.

Newsletter Going On-Line

Beginning with this issue, the Axolotl Newsletter will now be available on-line. To access the newsletter, just go to the Axolotl Colony website (<http://www.indiana.edu/~axolotl>) and choose The Axolotl Newsletter from the main menu. The on-line issue will be substantially the same as the paper copy.

We encourage all of you with access to the World Wide Web to read future issues of the Newsletter on the web. Anyone who would prefer to no longer receive the paper copy should send me an e-mail message to that effect (duhon@indiana.edu). I will notify all of