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Final Report

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Grant title: Lineage analysis of axis formation under novel gravity

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Grant Abstract: Recent intriguing work by Cooke ('86) and Neff, et al. ('93) suggests that there are subtle developmental changes in the Xenopus laevis embryos subjected to novel gravitational fields. These changes include the position of the third cleavage plane, the dorsal lip of the blastopore, and also the size of the head and eyes. However, compensation occurred later in development, so that by the tadpole stages there is no apparent difference between experimental and control embryos. How these early morphological changes are corrected is not clear. Through this project, we plan to determine whether the distribution of cytoplasmic morphogenetic determinants, and thus the developmental fate of blastomeres, is altered by novel gravitational fields by either tilting them or rotating them in a horizontal clinostat. We then plan to compare the control and experimental embryos with respect to blastomere fate (by lineage tracing with fluorescent dextrans), blastomere commitment and autonomous differentiation potential (by transplantation and culture), and distribution of cytoplasmic morphogens (by in situ hybridization). These three approaches, when applied in tandem, will provide a definitive test of the hypothesis that the distribution of cytoplasmic morphogenetic determinants and thus the developmental fate of blastomeres can be altered by novel gravitational fields.

Summary of research:

My NASA grant NAG5-5160 started June 1, 1995.

In the first year, we conducted Experiment I proposed in the grant. The question asked was: "Is cell fate changed under novel gravitational fields and is this change responsible for the morphological changes?". The results showed that cell fate is changed under novel gravity. We studied the cell fate change of all the blastomeres at the 8-cell stage and some blastomeres at the 32-cell stage in embryos which were subjected to 90° rotation before the first cleavage. We found that at the 8-cell stage, the blastomeres changed their normal fate; blastomeres on the top of the embryo always contribute to the rostral-dorsal part of the tadpole and those on the bottom contribute to the caudal-ventral part of the tadpole. At the 32-cell stage the blastomeres adapt fates according to new positions. However, the complicated pattern of the fate changes does not simply reflect a cytoplasmic shift after rotation, but it may be a combined effect of cytoplasmic reorganization caused by novel gravity and sperm activation.

In the second year we started the experiment II as originally proposed; that was to determine if the cell fate change in the rotated embryos is cell autonomous or cellular interaction dependent, using cell transplantation and cell culture techniques. The cell transplantation part of the experiments (IIb) was completed in the second year. The results demonstrate that blastomeres change their inducing and responding abilities in rotated embryos. The results from the cell culture experiments (IIa) also showed that blastomeres may change morphological pattern formed in culture after rotation of the eggs during first cleavage. Both experiments

indicate that changes in the rotated embryos may be caused by the reorganization of the cytoplasmic components, particularly the relocation of the dorsal determinants, under the effect of gravity.

In the second year we also started the experiments with horizontal clinostat which simulates microgravity conditions. The fate change from this experimental procedure is not as obvious as from the rotation procedure. However, the morphological alterations have already been shown from horizontal clinostat treatment and the investigation into the subtle fate change under microgravity conditions helps to elucidate the mechanism of the morphological alterations under microgravity, such as relocation of the maternally derived dorsal determinants.

In the third year, we completed the Experiment II and started and finished the Experiment III as proposed in the grant proposal. In addition, we performed some pioneer experiments that are related to the project, but not originally proposed.

1. Experiment II was designed to investigate the change in the autonomous differentiation capabilities of cultured blastomeres and the change in the ability of blastomeres to signal and respond during early inductive events . Normally, at the 16-cell stage, only dorsal blastomeres are able to elongate and express dorsal differentiation in explant culture. However, in 90° rotated embryos, the dorsal animal blastomere lost the ability to elongate or to express dorsal markers, while the ventral vegetal blastomere assumed these abilities. More importantly, the vegetal blastomeres in 90° rotated embryos and the dorsal blastomeres in clinostat-treated embryos increased their ability to elongate and express dorsal markers. It suggests that 90° egg rotation or microgravity may reorganize the cytoplasmic components, bringing localized dorsal determinants together, and thus facilitating the expression of dorsal fate in the blastomere. To study the possible molecules brought into the blastomeres to enhance their dorsal fate expression was proposed in my failed grant competitive renewal .

2. In Experiment II we also used cell transplantation to examine the effect of gravity on the inducing and responding abilities of blastomeres. As expected, these two cell properties are changed in 90° rotated embryos. That is, the dorsal inducing ability of normal dorsal blastomeres is adopted by ventral vegetal blastomeres in 90° rotated embryos. On the other hand, the vegetal blastomeres changed their ability to produce retina in rotated embryos..

3. We conducted the Experiment III. It included the in situ hybridization studies on the distribution of putative dorsal determinants, such as Vg1 mRNA, in normal and gravity-treated embryos. We performed in situ hybridization in clinostated, rotated and centrifuged embryos. The results demonstrate that Vg1 mRNA partially shift position under the effect of gravity, but the message mainly stays in the original location. Thus, gravity does not seem to influence the distribution of localized maternal RNAs.

4. The proposed in situ experiments with animal cap specific genes were not carried out as proposed. The major reasons are: 1) all the animal cap specific

genes (An-1, -2, -3) were not studied as thoroughly as the vegetal genes such as Vg1 and their restricted animal location was not well documented. 2) the distribution of RNA shown by in situ hybridization does not necessarily reflect the distribution their protein. Thus, the in situ results may not be very informative. 3) β-catenin is now being considered as an important and maybe animally located molecule which play a major role in dorsal axis formation. Thus, we started some work on β-catenin, instead of continuing the proposed in situ work of other animal genes. As recent studies suggested the importance of interaction between dorsal determinants on the dorsal differentiation. The idea of determinants interaction may explain why the dorsal differentiation of blastomeres is enhanced in 90° rotated embryos we observed. Therefore, we centrifuged normal and UV-treated embryos combined with injection of β-catenin and Vg1 RNAs to test the possibility of dorsal determinant interaction in dorsal differentiation. Some interesting results have been obtained and the analysis is still under way.

Publications:

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