Changes in the Cell Cycles During Early Embryogenesis of the Mexican Axolotl (*Ambystoma mexicanum*)

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In 1966 Graham and Morgan published the results of their detailed autoradiographic analysis of cell cycle changes during the early embryogenesis of *Xenopus laevis*. Since this aspect of urodele embryogenesis remained unexplored, I have performed an autoradiographic investigation of cellular proliferation in the early development of the Mexican axolotl (Desnitski, 1974a, 1974b). The cell cycle has been analysed in different regions of the embryo at various stages of development from the midblastula to the midneurula stage. The generation time (T or cell cycle duration) and the durations of the separate phases (G_1, S, G_2, and M) have been determined under continuous labelling conditions after injection of ^{3}H-thymidine.

The results of the study are summarized in Table 1. They show that durations of the cell cycle phases vary specifically with stage and region. The period of blastulation (Harrison stages 8 and 9) is characterized by the absence of the G_1 phase in animal cells, whereas the G_2 phase lasts 1.2-1.6 hours (18.8-23.1% of the cell cycle duration). In vegetative cells the G_1 and G_2 phases last 0.8-1.0 hours (8.4-14.3%) and 1.7-1.9 hours (20.0-24.3%) respectively. These phases change significantly in the course of subsequent development, and in various regions of the midneurula (Harrison stages 14-15), the G_1 phase lasts 10.1-15.2 hours (21.2-36.5% of the generation time), whereas the G_2 phase duration is only 4.5-5.4 hours (8.5-12.9%). Thus the relative duration of the G_1 phase tends to increase and that of the G_2 phase tends to decline during embryogenesis. The phase of DNA synthesis (the S phase) undergoes a 6- to 11-fold lengthening during development from midblastula to midneurula: from 3.2-3.4 hours (48.6-61.3% of the cell cycle duration) to 20.2-36.9 hours (48.4-66.4%). Changes in the duration of mitosis (the M phase) are insignificant when compared with those of the other phases of the cell cycle. The generation time, like the S phase, undergoes a 6- to 11-fold lengthening: from 5.2-7.0 hours at midblastula to 41.7-55.6 hours at midneurula. The cell cycle duration increases in the course of embryogenesis mainly by the lengthening of the S phase.

Thus the results indicate that the G_1 is the most variable phase in the cell cycle during the early development of the Mexican axolotl. This is in agreement with Signoret's (1980) investigation. According to his autoradiographic data, the cell cycle of the synchronously dividing axolotl embryo lacks any G_1 phase. On the other hand, the subsequent period of asynchronous division (blastulation) is characterized by a considerable variability in generation time in individual cells of the animal hemisphere; probably some cells may have a transient G_1 phase (Signoret, 1980). There is no obvious discrepancy with my evidence, because I have determined the mean durations of the cell cycle and its phases for the whole anlage, but not for individual cells.

Proceeding to a brief discussion of the specific traits of cell cycles at the stages of gastrula and neurula, it should be noted that my investigation cannot be reconciled with the autoradiographic data on *Xenopus laevis* (Graham and Morgan, 1966) and *Rana pipiens* (Flickinger et al., 1970). According to the results of both studies, the S phase occupies a comparatively small part of the cell cycle. However, these two studies are contradictory, too: Graham and Morgan have shown that the G_2 phase is the longest in the cell cycle during anuran gastrulation and neurulation, whereas Flickinger and coworkers have suggested that the longest is the G_1 phase.

On the other hand, the results of cytophotometric studies of the cell cycle in embryos of *Triturus vulgaris* (Lohmann, 1974) and *Rana temporaria* (Kovtunovich, 1969) are in good agreement. It has been shown in both papers that cells of gastrulae and neurulae spend the highest portion of the generation time in the S phase (up to 75-80% of the interphase). These studies are in accord with my autoradiographic observations on axolotl development. Therefore, there is reason to believe that, in their essentials, the principal trends in cell cycle changes during early embryogenesis are rather similar in several species of both urodele and anuran amphibians.

A new and interesting aspect of cell cycle analysis during early amphibian development

Table 1. Duration of the cell cycle (T) and its phases (hours) at different stages of the Mexican axolotl development

Regions	Harrison stages	T	G_1	S	G_2	M
Ectoderm	8	5.2	0	3.2	1.2	0.8
	9	8.2	0	5.5	1.6	1.1
	10	19.2	1.0	13.2	3.2	1.8
	10 1/2	18.5	0.9	14.3	2.1	1.2
Neural plate	14-15	55 .6	11.8	36.9	4.8	2.1
Epidermis	14-15	41.7	15.2	20.2	5.4	0.9
Chordamesoderm	14-15	52.6	12.0	34.6	4.5	1.5
Endoderm	8	7.0	1.0	3.4	1.7	0.9
	9	9.5	0.8	5.8	1.9	1.0
	10	22.7	3.6	12.3	5.3	1.5
	10 1/2	29.4	6.9	15.3	5.6	1.6
	14-15	47.6	1O.1	31.2	4.9	1.4

has been proposed in the autoradiograpic work of Maleyvar and Lowery (1973). They have shown that waves of mitosis and DNA synthesis pass through the presumptive neurectoderm during gastrulation in *Xenopus laevis*. The waves travel in synchrony with the invagination of the subjacent mesoderm, so they might be significant for the process of embryonic induction. The brevity of the cell cycle in *Xenopus* gastrulae did not permit the detection of a temporal or spatial distinction between the waves of mitosis and DNA synthesis. In principle, it would be reasonable to suppose that separate waves of the G_1 and G_2 phases pass through the neurectoderm as well.

So it was of interest to verify the data of Maleyvar and Lowery using slowly developing *Ambystoma mexicanum*. For this purpose I have determined the distribution of the S phase and mitotic indices along the presumptive neurectoderm of the axolotl at two stages of gastrulation (Harrison 10 1/2 and 11 1/2) (Desnitski, I978). However, at both stages the values of labelling and mitotic indices practically did not vary in different parts of the presumptive neurectoderm. On the other hand, the dorsal blastoporal lip was characterized by a very low S phase index as compared with the other rudiments. Nevertheless, a trivial explanation cannot be excluded that ^{3}H-thymidine poorly penetrates into this region. Decisive evidence concerning existence or lack of any waves of DNA synthesis and other cell cycle phases passing through amphibian gastrulae may be obtained after a detailed cytophotometric analysis.

In conclusion, it is appropriate to note that it would be of interest to investigate cell cycle changes during development of axolotl eggs spawned by *o/o* females.* These embryos always arrest at gastrulation. Thus the mutation might provide some information towards elucidating the role of the cell cycle in early urodele differentiation.

*Unfortunately, the *o* gene has not been seen for many years in the Axolotl Colony (editor).

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