The Induction of the Primordial Germ Cells in the Urodeles

Lien A. Sutasurja and P. D. Nieuwkoop
Hubrecht Laboratory, Utrecht, Holland

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Summary. Xenoplastic recombinates of animal ectodermal caps with the ventral vegetative yolk mass of blastulae of stage 81/2-83/4 of *A. mexicanum*, *T. alpestris*, *T. cristatus carnifex* and *P. wallii* have demonstrated unambiguously that in the urodeles the primordial germ cells—together with other ventro-caudal mesodermal structures—develop from the animal ectodermal moiety of the blastula under an inductive influence emanating from the ventral vegetative yolk mass. Similar recombinates of 3H-labeled and unlabeled ectodermal and endodermal components fully support this conclusion.

Recombinates of the ventral vegetative yolk mass with different regions of the animal ectodermal hemisphere show that primordial germ cells can be formed by any region of the animal ectodermal hemisphere, including those regions which in normal development will never form them. The number of primordial germ cells formed differs significantly among the various regions, that of the ventral peripheral region being the highest and that of the central, animal region the lowest. The capacity for primordial germ cell formation shows two increasing gradients, one animal-vegetative and the other dorse-ventral (in the peripheral zone). Although accurate measurements could not be made, there seems to be a relation between the number of primordial germ cells formed and the amount of ventro-caudal mesoderm induced.

The experiments, moreover, show that notochord differentiation largely or entirely suppresses primordial germ cell formation. Notochord differentiation shows a similar animal-vegetative, but an opposite ventro-dorsal increase in frequency (in the peripheral zone) as compared with the capacity for primordial germ cell formation. The notochord-forming gradient in the peripheral regions is mainly due to the inductive action already exerted by the dorsal vegetative yolk mass in the intact blastula prior to isolation and recombination (see control explants). The ventro-dorsal decline in primordial germ cell formation in the peripheral regions is very probably due only to the inhibition of primordial germ cell formation by notochord differentiation (as an expression of dorsal mesoderm induction). Therefore, in the animal ectodermal moiety of the blastula there exists only an animal-vegetative gradient in mesodermal competence.

These results make it very likely that in urodeles the primordial germ cells do not arise from predetermined elements such as those demonstrated in anurans, but develop from common, totipotent animal ectodermal cells. The discrepancy in the mode of origin of the primordial germ cells between anurans and urodeles could be due only to pronounced differences in the time of appearance of the germinal cytoplasm (in anurans during oogenesis, in urodeles possibly during determination of the primordial germ cells within the ventro-caudal mesoderm).

The differences in site and mode of origin of the primordial germ cells between urodeles and anurans favor a dual phylogenetic origin of the two groups.

When anurans and urodeles are compared, a rather fundamental difference in origin of primordial germ cells seems to exist. In the anurans descriptive and experimental studies indicate almost unanimously an early, endodermal origin (see Blackler, 1970), whereas in the urodeles the majority of experimental studies suggest a later, mesodermal origin (Humphrey, 1929; Nieuwkoop, 1947; Smith, 1964; Capuron, 1968, 1972). Apart from the difference in site of origin there seems
to be a more fundamental divergence in mode of origin. In the anurans an uninterrupted "germ line" has been traced back from the adult to the fertilized egg (Bounoure, 1939) and even to the ripe ovarian oocyte (Czołowska, 1969); it is characterized by the presence of special cytoplasmic inclusions, the so-called "germinal cytoplasm". This becomes localized in particular cells during cleavage as a result of unequal divisions (K. E. Dixon, personal communication). In the urodeles the primordial germ cells could so far only be traced back to an early tail bud stage, when, according to Humphrey (1925), they are recognizable by their light color in the dorso-caudal part of the lateral plate mesoderm.

Although some contradictions in the literature seem to be due to insufficiently critical experiments, others find their origin in the fact that experiments were carried out at different stages of development. Asayama (1950, 1961), Asayama and Amanuma (1957), and Amanuma (1957, 1958) concluded from ablations of presumptive lateral plate mesoderm at tail bud stages that in Triturus pyrrohaster and Hynobius nebulosus the primordial germ cells can still arise from somatic cells in relatively advanced stages of development. These observations were recently tested by Capuron (1972) who confirmed Humphrey's (1927, 1928, 1929) conclusions and disproved those of the Japanese authors. Kotani (1957, 1958) replaced presumptive lateral plate mesoderm by presumptive ectoderm in early T. pyrrohaster gastrulae and found no effect on the number of primordial germ cells in further development. From this he also inferred a somatic origin of the primordial germ cells in the urodeles, a view which seems to contradict Nieuwkoop's (1947) conclusion that the primordial germ cells in the urodeles originate exclusively from the presumptive lateral plate mesoderm of the middle gastrula (yolk-plug stage). Recently Kotani's conclusion received strong support from Kocher-Becker and Tiedemann (1971) who described the induction of primordial germ cells—together with mesodermal and endodermal structures—in Triturus early gastrula ectoderm by means of a purified vegetalizing factor isolated from chick embryos. This apparent contradiction finds a satisfactory explanation, however, in the results of Nieuwkoop's recent mesoderm induction experiments (Nieuwkoop, 1969a, 1969b, 1970, 1973; Nieuwkoop and Ubbels, 1972) in which it was demonstrated that the entire mesoderm develops epigenetically, that is, that it is induced in the animal ectodermal moiety of the blastula by the endodermal vegetative yolk mass. In the case of induction of ventro-caudal mesoderm, which normally occurs under the influence of the ventral portion of the vegetative yolk mass, primordial germ cells constitute one of the typical elements of the induced mesoderm (Boterenbrood and Nieuwkoop, 1973). The mesodermal competence of the animal ectodermal moiety sharply declines during the first phase of gastrulation (Leikola, 1963, 1965). Kotani's experiments were therefore carried out with ectoderm which was still competent, whereas Nieuwkoop's experiments were done after determination of the mesoderm and primordial germ cells had already taken place. The negative result of one of Smith's (1964) experiments, that in which lateral mesoderm of a middle gastrula was replaced by presumptive ectoderm from an early gastrula, can be similarly explained by assuming that the endoderm

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1 The term "ectodermal" is commonly used for the animal moiety of the blastula; strictly speaking it is only applicable to a stage of development in which germ layers have been formed. The same can be said for the term "endodermal" for the vegetative moiety of the blastula.