Changes in DNA and RNA during Embryonic Development of the Merogonic Combination *Triton palmatus* (♀) × *Triton cristatus* (♂)[1]

In a number of previous studies BALTZER[2-4] and HADORN[5-7] investigated in detail the morphogenetic capacities of the merogonic combination *Triton palmatus* (♀) × *Triton cristatus* ♂. According to these two authors, the (p)c merogone develops at best to an early embryo with neural tube and eye vesicles, and then dies with a localized pycnosis in the head mesenchyme. We know, however, very little about the biochemical properties of this androgenetic haploid hybrid. ZELLER[8] carried out an extensive study on the synthesis of ribonucleic acid (RNA) in the (p)c embryos. He showed that there is a rapid increase in RNA during the development of the merogone. Obviously the *cristatus* nucleus is able to synthesize RNA, which, however, cannot be utilized by the *palmatus* cytoplasm. Changes in desoxyribonucleic acid (DNA) were not investigated.

Very recently, by using a modified microchemical method, CHEN[9] analyzed the synthesis of both DNA and RNA during the normal embryonic development of *T. alpestris*, *T. palmatus*, and *T. cristatus*. In general, his results revealed that the DNA content keeps a constant value until the late blastula stage and new synthesis begins shortly before gastrulation; during gastrulation and neurulation, the DNA increase is especially rapid. A similar situation was found for RNA: its synthesis does not take place until the end of blastulation. The increase in RNA during gastrulation and neurulation is, however, slow compared to that in DNA.

The microchemical method described by CHEN[9] is sensitive enough for estimating the nucleic acids in half an embryo. The procedure involves the differential extraction of DNA and RNA by perchloric acid and the contents determined by spectrophotometry using micro-attachments for Beckman model DU. It would be of interest to follow the synthesis of both nucleic acids in the (p)c merogones by using this new method. The main point here is to find out whether the *cristatus* nucleus can make use of materials in the cytoplasm of another species to form new DNA and RNA. Since haploid hybrids which reach advanced stages are very rare, our data are limited, and the present paper should be considered as a preliminary report on this problem.

The technique of obtaining merogonic hybrids was similar to that described by previous authors (BALTZER[2-4], HADORN[5-7], and ZELLER[8]), except that for enucleation, a fine needle was inserted into the glass micropipette so that the unfertilized *palmatus* egg could be pricked and the egg nucleus removed in the same procedure (Fig. 1). Embryos of desired stages were cut into two equal sagittal halves, the one half being used for the chemical analysis and the other half squashed on a slide and stained with orcein-acetic acid for counting the chromosomes. In addition to hybrid embryos identified as haploid, individuals with the diploid number of chromosomes were also found; these apparently were due to an unsuccessful enucleation. We shall refer to these embryos as operated diploid pc hybrids (op.pc), while those which had not been subjected to the enucleation procedure will be termed normal diploid pc hybrids (nor.pc). Both types of embryos served as controls. The developmental periods chosen for the nucleic acid analysis were as follows: (1) late blastula to early gastrula aged about 25–34 h after fertilization, (2) early to late neurula at 60–78 h of age and (3) early embryo with neural tube and eye vesicles at 85–98 h of age. We have examined altogether 68 haploid and diploid

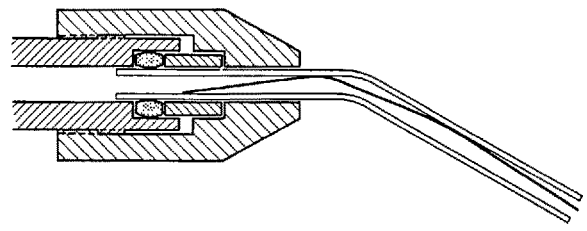

Fig. 1. Glass micropipette with a fixed fine needle for pricking the egg and removing the egg nucleus in the same procedure. The micropipette is fastened to a metallic holder (shown by the hatched part in the sketch) used in the transplantation technique for *Drosophila*. The holder is connected to a hypodermic syringe by a plastic capillary tubing (not shown in the Figure). The whole apparatus is filled with water during operation. By manipulating the syringe, the egg nucleus together with some cytoplasmic material can be sucked into the micropipette immediately after pricking.

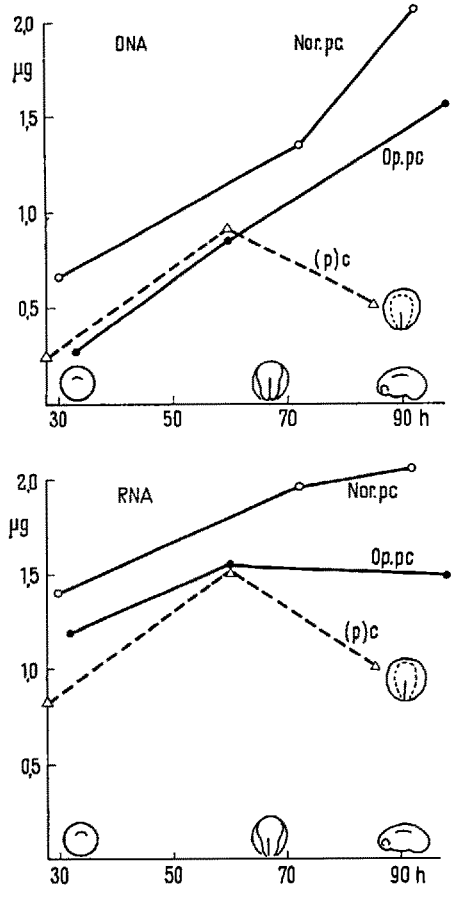

Fig. 2. Pattern of DNA and RNA synthesis in haploid (p)c merogones (△), normal (○), and operated (●) diploid pc hybrids. Ordinate: nucleic acid content (µg/½ embryo). Abscissa: developmental age in h after fertilization at 18° C.

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embryos. Among these only 10 merogones, 18 normal and 6 operated pc hybrids were found to be healthy and could be used for evaluation of the DNA and RNA data. The number of chromosomes in these embryos could be estimated with certainty.

Results. Our over-all data on DNA and RNA are presented in the Table and the average values for the three developmental periods mentioned in the previous section are summarized in Figure 2. As can be seen from the Table there are certain variations in the nucleic acid contents in embryos of about the same age, but the general pattern of DNA and RNA increase in the three types of embryos is evident. In the normal diploid pc hybrids (nor.pc), there is a continuous increase of DNA from late blastula until the early embryo stage (Fig. 2). Considering that the development of this hybrid combination is normal, this increase is understandable. In the previous study of CHEN[9], it has been shown that the DNA content of both parental species also rises rapidly during the same developmental period. The operated diploid pc hybrids (op.pc) show the same pattern of DNA increase, although its absolute amount is distinctly lower than that in the normal hybrids. It should be mentioned that during enucleation a part of the egg cytoplasm was removed. The operated embryos are thus smaller than the non-operated pc controls. Also, from earlier studies, it is known that in the amphibian egg there is a large cytoplasmic store of DNA (for literature see CHEN[9]). This seems to be the reason why the absolute quantity of DNA is lower in the operated diploid hybrids. In the hybrid merogones, there is a distinct increase of DNA from late blastula to mid-neurula stage. However, after this stage there seems to be no increase in the DNA content. To what extent this is due to cell degeneration, during later development, is uncertain. As shown by the curves in Figure 2, during the period from late blastula to mid-neurula the haploid (p)c merogones have about the same amount of DNA as the operated diploid pc hybrids. Considering that the nuclei of the diploid individuals contain twice the number of chromosomes as the haploid ones, this result is not to be expected. We have counted the number of nuclei in sections of one pc diploid and one (p)c merogonic embryo. This showed that these are on the average 4.94 nuclei per unit area in the pc control and 8.43 in the (p)c merogone. Apparently there is an adjustment in nuclear number, compensating for the decrease in nuclear size. In anurans HERTWIG[10] found twice the normal cell number in haploid gastrulae. Very recently, such a regulation was also recorded for diploid and triploid individuals of the fish *Gasterosteus aculeatus* (SWARUP[11]). The problem of the relation of cell number to cell size has been discussed in detail by FANKHAUSER[12].

Age, development and nucleic acid contents of haploid (p)c merogones, operated (op) and normal (nor) diploid pc hybrid embryos

Embryo type	Age in h after fertilization	Development[a]	RNA ($\mu g/^1/_2$ embryo)	DNA ($\mu g/^1/_2$ embryo)
(p)c	25.5	8+/H/N	0.85	0.39
	25.5	8+/H(+)/N	0.96	0.42
	27	8+/H/N	0.76	0.12
	27	8+/H(+)/N(±)	0.86	0.26
	27	8+/H(+)/N	0.80	0.24
	27	8+/H/N	0.68	0.24
	34	9–10/H/N(±)	0.88	0.12
	60	12c/H(+)/S	1.47	0.77
	60	13/H/N(±)	1.59	1.01
	85	13/H/S	1.06	0.51
pc(op.)	33	11a/D/N	1.23	0.35
	33	11a/D/N(±)	1.14	0.18
	60	13/D(+)/N(±)	1.63	0.92
	60	13/D(−)/N	1.46	0.81
	98	15/D/N	1.31	1.28
	98	21/D(+)/N	1.59	1.86
pc(nor.)	25.5	8+/D/N	1.26	0.72
	27	8+/D/N	1.37	0.92
	27	8+/D/N	1.10	0.43
	33	9–10/D/N	1.84	0.49
	33	9–10/D/N	1.88	0.65
	34	11b/D/N	1.10	0.74
	34	11b/D/N	1.28	0.69
	60	15/D/N	1.82	1.40
	60	15/D/N	2.04	1.25
	78	15/D/N	1.96	1.34
	78	15/D/N	1.96	1.33
	78	15/D/N	1.94	1.27
	78	15/D/N	2.16	1.59
	85	23/D/N	2.52	2.22
	85	23/D/N	2.02	1.83
	94	21/D/N	1.73	1.81
	98	19/D/N	2.29	2.88
	98	19/D/N	1.73	1.72

[a] The numbers in this column refer to Harrison's developmental stages for *Amblystoma punctatum*. H and D indicate haploid and diploid number of chromosomes respectively. '+' and '−' show that small variations in the number of chromosomes were counted. *N* means that the developmental state of the embryo was normal, while S indicate that sticky, possibly degenerated, cell materials were observed in the inside of the embryo.

The pattern of RNA synthesis for the three types of hybrids is also shown in Figure 2. In the normal pc hybrids (nor.pc), there is a gradual increase of RNA from late blastula to embryo with eye vesicles. However, the magnitude of increase is less compared with that of DNA, especially at later stages. This difference was also found for the two parental species (CHEN[9]). In a similar way the operated pc hybrids show a steady increase in RNA, but the total quantities are again markedly lower than those of the normal diploid pc hybrids at corresponding stages, apparently again due to removal of egg cytoplasm during enucleation. In the hybrid merogones we observed a definite increase of RNA during gastrulation and neurulation. Our data indicate that at the midneurula stage the (p)c embryos are at least as rich in RNA as the operated pc individuals. This result confirms the earlier findings of ZELLER[8] that active synthesis of RNA takes place during the early development of the haploid androgenetic hybrid. Only at about 90 h of age, its RNA content seems to be lower. Further work is needed to ascertain how far the inhibition of RNA formation at such a later stage is due to a general impairment of morphogenetic development of this merogonic combination.

The present study demonstrates that, at least up to the midneurula stage, the *cristatus* nucleus is able to utilize precursors in the *palmatus* cytoplasm to synthesize both DNA and RNA.

Zusammenfassung. Der Gehalt an RNS und DNS früher Entwicklungsstadien des Bastardmerogons *Triton palmatus* (♀) × *Triton cristatus* ♂ wurde gemessen. Es zeigt sich, dass trotz der Kern-Plasmadisharmonie diese letale Kombination in der Lage ist, beide Nukleinsäuren zu synthetisieren.

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