isolated, subjected to the routine histological procedure, and examined for the presence of mature tissues.

The results of the histological examination are summarized in the Table. It is obvious that, in the present experimental conditions, the two areas of the ectoderm do not significantly differ in their capacity to differentiate into the neural tissue and the epidermis and its derivatives. Quantitatively, the differentiation of neural tissue was predominant in grafts of both series. The possible explanations of this feature (to be further analyzed in a more extensive study) are: a) the cuts, by which the ectoderm was divided into 3 areas, did not correspond to the demarcation between the presumptive neurectoderm and the presumptive epidermis; b) the 2 developmental capacities of the ectodermal cells are not yet strictly regionally restricted at this stage; c) the competences of different areas of the ectoderm are not yet definitively stabilized; d) the atypical environment has led to the partial neuralization of the lateral areas of the ectoderm (presumptive epidermis?); e) different combinations of the above-mentioned possibilities.

We have previously shown that the head-fold stage rat embryonic ectoderm still contains some prospective mesodermal cells[3]. In the present experiment, mesodermal tissues appeared quite regularly in both series, although the grafted areas of the ectoderm were lacking the regions of in situ immigration of prospective mesodermal cells (primitive streak, Hensen's node). This can be explained by at least 2 possibilities: a) some cartilage and bone may have originated from the neural crest cells ('mesectoderm'); and b) in experimental conditions, the prospective mesodermal cells can segregate and differentiate in any area of the ectoderm ('regeneration' of the primitive streak).

Cytochemical Evidence for Stage-Specific Changes of Nuclear RNA and Nonhistone Protein Content During Early Development of *Triturus vulgaris*

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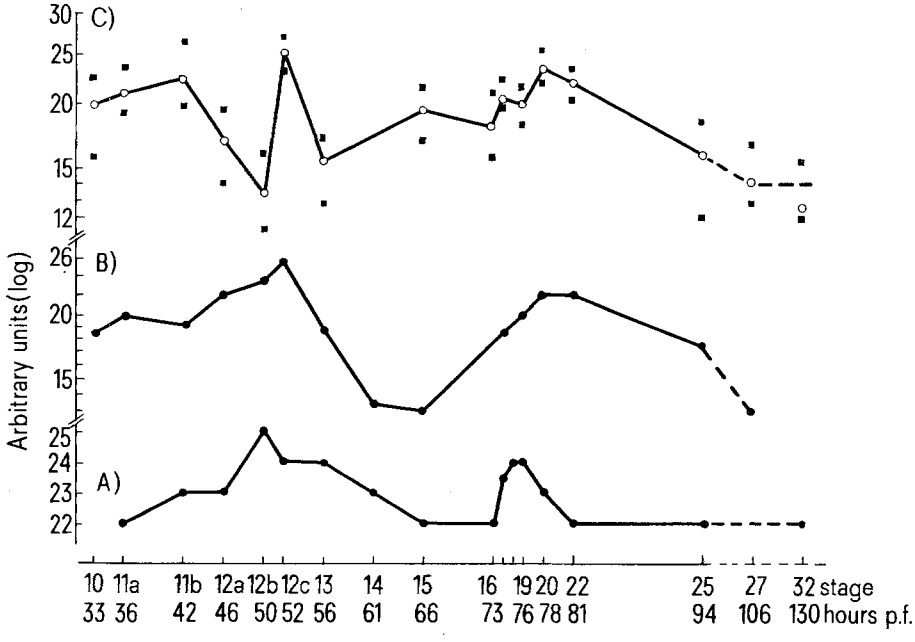
Summary. During early embryogenesis of *Triturus vulgaris*, RNA and nonhistone protein contents of neuroectoderm nuclei change with stage specifically. Maximum values were found in the late gastrula after embryonic induction, and in the late neurula with the formation of the neural tube. The stage-specific increases of RNA and nonhistone protein are correlated with a preceding increase of Feulgen-DNA content.

In recent years much evidence has been accumulated to show that nonhistone proteins participate in regulation of transcription[1]. On the other hand, studies on synthesis and turnover of nuclear precursor-RNA led to new hypotheses and models about the mechanism of gene expression in eukaryotic cells[2].

From cytophotometric measurements of Feulgen-DNA content in various regions and developmental stages of *Triturus vulgaris*, which did not agree with the theory of DNA constancy, we made the assumption that the phase-specific increase of DNA might cause an enhancement of gene activity in definite regions of the embryo[3–5].

Materials and methods. From small pieces of neuroectoderm (from early gastrula to tailbud) nuclei smears were prepared as described earlier[4]. Nonhistone proteins were stained for 30 min with 0.1% fast green FCF at pH 2.0, according to the procedure of KAYE and MCMASTER-KAYE[6]. Before staining nucleic acids were removed with hot TCA (5%, 90 °C, 15 min), and then histones were extracted by immersion of the slides in 0.1 *N* HCl



Changes of DNA (curve A), RNA (curve B) and nonhistone protein (curve C) content of neuroectoderm nuclei during development of *Triturus vulgaris*. Ordinate: relative dye content in arbitrary units. Abscissa: developmental stages (Harrison) and h after fertilization (18 °C). The points in curve C illustrate the medians with the 95% confidence limits.

for 24 h at 32 °C. Nucleic acids (DNA and RNA) were stained by methylene blue (Merck, Darmstadt; C. I. Nr. 52015; PISCHINGER[7,8]; 0.002 *M*, pH 4.8, 15 min). After RNase treatment (2 mg/ml, 20 U, 3 h, 37 °C) the DNA: RNA ratio of nuclei of differentiated cells (mesencephalon, stage 32) was estimated to be approximately 1:1. On the basis of the Feulgen-cytophotometric measurements of DNA content[4] and the described DNA/RNA ratio, the RNA content of nuclei could be calculated by measuring the total nucleic acid content. For determining the nucleic acid and nonhistone protein content, the stain intensity was measured in 70 individual nuclei from each slide with a Barr and Stroud integrating microdensitometer (Glasgow, Type GN 2).

Results and discussion. In all cases the measurement data, which were collated to so-called karyograms, show a doubling of dye content during the cell cycle. This means that nuclei double their RNA and nonhistone protein content from G_1 to G_2 phase, as is known for DNA and histone. Further, it became evident that the RNA and nonhistone protein contents are not constant in the course of development, but vary with stage specifically. At the onset of gastrulation, no considerable changes in nuclear RNA content can be found until stage 11b (curve B). Then a remarkable increase occurs with a maximum in the late gastrula (stage 12c, about 96% over the definitive value). In the following stages, the RNA content decreases rapidly and reaches minimum values with the formation of the neural plate (stage 14, 15). A second drastic increase in nuclear RNA content (about 70%) can be detected in the late phase of neurulation, followed by a continuous reduction to the tailbud stage.

These changes in nuclear RNA content are correlated with significant changes in the Feulgen stainability of nuclei in the appropriate stages (curve A). The comparison of the 2 curves (A, B) shows that in both phases the DNA content reaches maximum values first, to be followed by the RNA maximum about 2 h later. There is a good temporal correlation between the increases of DNA and RNA during gastrulation. During neurulation, the accumulation of RNA extends over the whole period, whereas the additional DNA increase is of short duration. These results support our assumption that the stage- and region-specific increases of Feulgen dye content[4] are an expression of enhanced gene activity during early development.

The changes of nonhistone protein content in the course of development are illustrated in curve C. With the beginning of gastrulation, the nonhistone protein content increases slightly and is then rapidly diminished from stage 11b to 12b. Because it is not possible with cytochemical methods to distinguish between chromosomal and non-chromosomal proteins, the measurement data always represent the changes of the sum of both protein classes. By measuring nuclear sizes (JANSEN, unpublished results), it was found that nuclear volume decreases continuously from stage 10 to 12b. From this fact, we should also expect a continuous reduction of nuclear protein content, as is shown for stage 11b to 12b. Assuming that the decrease of nuclear size is due to a loss of non-chromosomal protein, the slight increase of nonhistone protein indicates an accumulation of chromosomal proteins prior to the beginning of RNA synthesis (curve B), which is in agreement with findings that nonhistone chromosomal proteins are responsible for tissue-specific gene activation[9,10]. At a time when RNA content shows maximum values, a drastic increase in protein content (about 90%) takes place and is correlated with a spontaneous swelling of nuclei. This confirms the generally accepted idea that nucleic acids always occur in close association with protein. The parallel decrease of RNA and protein is, therefore, probably caused by the transport of ribonucleoprotein from nucleus to cytoplasm. During neurulation, similar correlations between RNA and protein content exist. Before the onset of RNA synthesis, the nonhistone protein content of the early neurula is increased. At the end of neurulation, RNA and protein have highest values which were slowly reduced in the following stages to the final amount of the tailbud stage.

The temporal correlation of our results with early embryonic induction on one hand, and the differentiation of the neural tube on the other, raises the question of what kinds of RNA are synthesized, and whether there are stage-specific changes in the nonhistone protein pattern during gastrulation and neurulation. The fact, however, that the stage-specific increase of RNA is accompanied by a preceding synthesis of additional DNA focusses attention on the possibility of the existence of gene amplification during early development. This point is now under investigation.

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Coated Vesicles in the Rat Adrenal Glomerular Zone After a Low-Sodium Diet

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Summary. In rats subjected to a low-sodium diet, a great activity was observed of the coated vesicles at Golgi complex and cell surfaces of glomerular adrenal zone. These findings are related to the function of these organoids in the uptake and transport of necessary substances under stimulating conditions of the zone.

The presence of coated vesicles in the adrenal cortex cells of various species has been confirmed in recent investigations[1-5]. In these previous studies these elements were discovered in relationship with the Golgi complex and the cellular membrane. It is recognized that these vesicles participate in the transport of protein substances

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