Investigation of the mode of nuclear control over protein synthesis in early development of loach and sea urchin

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SUMMARY

It is shown that in loach embryos the incorporation of precursors into protein takes place in the blastoderm cells only. The change of the rate of incorporation of labelled amino acids into protein of the blastoderm separated from the yolk at successive developmental stages reflects the changes in the level of protein synthesis in intact embryos of the same developmental stages. Typical periodic changes of the intensity of protein synthesis in early embryogenesis of the loach are detected: low incorporation of amino acids at blastula stages is followed by an increase of synthesis during gastrulation and by a decrease with the onset of organogenesis.

To study the genetic control over protein synthesis at various developmental stages of loach and sea-urchin embryos the effects of ionizing radiation and long-term treatment with actinomycin D have been examined. X-Irradiation doses produce an insignificant direct effect on protein synthesis, while radiation damage of the nuclear apparatus results in a gradual but ever increasing inhibition of protein synthesis. The inhibition of RNA synthesis with actinomycin or ionizing radiation damage of the nuclei produce essentially the same effect on the intensity of protein synthesis. Protein synthesis in androgenetic haploid hybrid embryos (loach?×goldfish♂) and in loach androgenetic haploid embryos after producing a partial elimination of chromosomes does not differ from 'enucleated' loach embryos completely deprived of chromosomes. These data suggest that high-polymeric RNA formed after the elimination of some chromosomes is unable to provide a normal level of protein synthesis.

Protein synthesis is not controlled by the nuclei up to the stages of early blastula (sea urchin) and of late blastula (loach), being evidently programmed in oogenesis. To provide a rapid activation of protein synthesis in the course of gastrulation in the loach the function of the nuclei has to be realized during mid-blastula stages. An increase of the rate of the incorporation of amino acids at the stages of mesenchyme blastula in the sea urchin depends on the synthesis of RNA at the early blastula. At the same time protein synthesis during gastrulation in the loach and at mesenchyme blastula in the sea urchin is much less dependent on the simultaneous RNA synthesis. Protein synthesis at these stages seems to be provided by the long-living templates and controlled by non-gene mechanisms of the regulation of translation.

Thus early embryonic differentiation in the loach and sea-urchin development is related to the activation of protein synthesis. The latter is provided by the preceding morphogenetic nuclear function, which makes protein synthesis relatively independent of simultaneous synthesis of templates that ensures subsequent development stages.

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INTRODUCTION

A complicated control of protein synthesis is known to occur in early development. It proceeds on RNA templates stored in oogenesis, and on new long- and short-living ones synthesized in the nuclei of the embryo. The intensity of protein synthesis and possibly the selection of templates are controlled by translation mechanisms whose nature is mostly unknown. Recently a particular emphasis has been placed upon the special phenomenon of the sudden intensification of protein synthesis after fertilization of sea-urchin eggs. Little is known so far about protein synthesis in other species of animals or about protein synthesis at advanced stages of sea-urchin development.

Arrest of RNA synthesis after actinomycin D treatment (Gross & Cousineau, 1963) provides a method which allows the assessment of the contribution of direct gene control over protein synthesis. The present work was aimed at studying the contribution of the genetic apparatus by its inactivation at successive developmental stages by means of both actinomycin treatment and large doses of ionizing radiation. The latter has already been used to evaluate the participation of the nuclei in early embryogenesis of various species (Neyfakh, 1959, 1964). Besides sea-urchin eggs loach embryos were used – a convenient material which has long been used in this laboratory. Some additional data on the relationship between RNA and protein synthesis were obtained on lethal fish hybrids.

MATERIAL AND METHODS

The experiments were carried out on loach, Misgurnus fossilis, and sea-urchin, Strongylocentrotus nudus, embryos. The eggs were obtained from loach females 40 h after the injection of gonadotrophic hormone, choriogonin. Developmental stages of the loach were expressed in hours of development after fertilization at 21 °C according to Neyfakh (1959). The hybrid embryos were obtained by fertilizing loach eggs with goldfish sperm. Radiation inactivation of loach gametes and embryos at different developmental stages was performed on X-ray machine RUP-1 (180 kV, 15 mA, without filter).

To study the changes of protein synthesis in intact loach embryos and hybrid embryos, loach♀ × goldfish♂, portions of eggs were incubated in closed vessels with [14C]carbonate to give a final concentration of 50 µCi/ml in Flickinger solution (Flickinger, 1954). The isolation of blastoderms of the loach and hybrid of loach♀ × goldfish♂ embryos according to Kostomarova (Kostomarova, 1969) was performed immediately before the incubation with labelled amino acids, which was carried out in double-strength Holtfreter's solution. The latter was prepared with 0.05 M Tris-HCl buffer, pH 7.8, containing 100 i.u./ml of penicillin and 50 i.u./ml of streptomycin. The mixture of [14C]amino acids in a final concentration of 2 µCi/ml per sample was used as precursor.

Artificially fertilized sea-urchin eggs were incubated at a concentration of
5000–10000 eggs per ml under gentle manual swirling at 21–23 °C. The samples were X-irradiated on machine RUM-3 (15 mA, 180 kV). Actinomycin D was used at a concentration of 25 μg/ml, puromycin at 30 μg/ml. To determine the intensity of protein synthesis the samples of 10000 eggs were taken and incubated with [14C]amino acids at a concentration of 0.5 μCi/ml. Incubation was carried out in sea water filtered through RUF-2 filters containing penicillin (100 i.u./ml) and streptomycin (50 i.u./ml).

After incubation the eggs were fixed with 5 % TCA (trichloroacetic acid) containing an excess of cold amino acids, washed from acid-soluble fraction, nucleic acids and lipids by routine procedures. The residue was dissolved in formic acid, protein concentration being determined according to Lowry (Lowry, Rosenbrough, Farr & Randall, 1951), its radioactivity being counted in a Geiger counter. The specific radioactivity was expressed as impulses per minute per mg of protein.

The data on the incorporation of labelled amino acids into protein were calculated from protein activity in incubated samples minus the absorption value. To determine the absorption embryos were incubated with labelled amino acids in ice for the appropriate time and treated as all the other samples.

RESULTS

Study of protein synthesis in the loach embryos

Impermeability of loach eggs to water-dissolved substances has complicated the task. First, at all developmental stages except the earliest ones the nuclear function was inactivated with high doses of ionizing radiation rather than with specific inhibitors. This required a number of procedures to show that the effect of ionizing radiation on protein synthesis was like that of actinomycin. Secondly, to study protein synthesis in intact eggs, instead of amino acids, an unspecific labelled precursor [14C]carbonate was used.

Loach eggs at blastula and gastrula stages were incubated in Flickinger acid medium with a solution of [14C]carbonate. Then a portion of intact eggs was fixed, another portion of shell-free eggs was separated in sucrose gradient into
Fig. 1. [14C]carbonate and [14C]amino acids as precursors. At given developmental stages the eggs were incubated for 2 h with [14C]carbonate solution (50 μCi/ml). One portion was fixed immediately after the incubation and the specific activity of protein was determined (curve 1, scale I). Blastoderms were isolated from the second portion of eggs after their incubation with [14C]carbonate, and the specific activity of protein was determined (curve 2, scale II). At the same developmental stages blastoderms were isolated and incubated with mixture of [14C]amino acids, after that the specific activity of protein in them was determined (curve 3, scale III).

Specific activity of the total protein of the whole egg seems to result from at least a 10-fold dilution of blastoderm protein with unlabelled yolk protein (for the yolk mass is about 10 times that of the blastoderm mass).

When isolating blastoderms from yolk the surface bordering the yolk becomes naked. This surface being suitably permeable, the blastoderms were used for the incubation with labelled amino acids. Fig. 1 compares the intensity of protein...
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synthesis in intact embryos incubated with $[^{14}C]$carbonate, in blastoderms isolated from these embryos and in blastoderms incubated with $[^{14}C]$amino acids. The scales are so chosen that the values of the intensity of protein synthesis at the developmental stage of 14 h coincide on Fig. 1 for all three experimental series. The slopes of these curves also turned out to be close to each other.

The intensity of protein synthesis was also studied under the same incubation conditions in the eggs irradiated at the stage of late blastula. The change of the intensity of amino acid incorporation observed after irradiation is virtually the same in intact eggs, in embryos separated from the yolk incubated with $[^{14}C]$carbonate and in those isolated after the incubation with $[^{14}C]$amino acids. Similar changes of the intensity of protein synthesis in intact eggs (precursor $^{14}$CO$_2$) and in isolated blastoderms (precursor $[^{14}C]$amino acids) were also found for diploid and haploid embryos of the loach.

It seems possible to interpret the change in the rate of protein synthesis in terms of the incorporation of labelled amino acids into protein only if the specific activity of amino acid pool at the developmental stages studied remains constant. The specific activity of amino acids in the cell, when amino acids are added to the medium, depends both on the rate of their penetration into the cell, and on its pool in the cell. When $[^{14}C]$carbonate is used the problem of the change of permeability seems to be insignificant. The specific activity of intracellular amino acid pool in this case will depend on the rate of carboxylation (with the participation of biotin) of keto acids on the one hand and on the rate of amination on the other. In the course of development all these parameters may change. However, the similarity of the curves obtained applying various precursors suggests that, first, carboxylation of keto acids with formation of amino acids does not limit the rate of protein synthesis in loach embryos, and secondly, that the specific activity of intracellular amino acid pool is changed insignificantly in the course of development. It was also concluded after chromatographic analysis and direct determination of the specific activity of intracellular amino acid pool (Kukhanova & Neyfakh, unpublished, Neyfakh, 1971) that the latter is invariable in the embryos isolated at various stages of early loach development. Consequently the level of the incorporation of amino acids into protein during 1 h incubation of embryos isolated from the yolk at successive developmental stages reflects the intensity of their protein synthesis in each given moment of development. These results allowed further studies on protein synthesis in early development of the loach by the incubation of blastoderms with labelled amino acids.

The change in the intensity of protein synthesis in the course of development is presented in Fig. 2, showing the results of six experiments. The incorporation of amino acids into embryos' protein insignificantly increases during mid–late blastula, but with the onset of gastrulation the rate of protein synthesis rapidly increases. During the developmental period from mid-blastula up to the late gastrula the rate of protein synthesis increases 5–6 times. At subsequent developmental stages the intensity of protein synthesis decreases and reaches the
Fig. 2. Intensity of protein synthesis in the development of loach embryos (the data of six experiments). At given developmental stages blastoderms were isolated and incubated with mixture of $[^{14}\text{C}]$amino acids, and the specific activity of protein was determined.

minimum at the stages of 3–7 somites (22–24 h after fertilization). At this stage the intensity of the incorporation of amino acids amounts to one half of the maximum in early development at the stage of late gastrula and is equal to the rate of protein synthesis at early gastrula stage, 24 h after which the intensity increases again.

**Effect of irradiation on protein synthesis in loach embryos at various developmental stages**

As demonstrated above, the intensity of protein synthesis in loach embryos changes regularly, which allowed us to consider it as one of biochemical criteria of differentiation. To study the dependence of the intensity of protein synthesis on nuclear control, loach eggs were irradiated at various developmental stages at a dose of 20 kr (516 C/kg air) that completely inactivated the morphogenetic function of the nuclei (Neyfakh, 1959).

The inactivation of nuclei with heavy doses of ionizing radiation required a study of possible damage to the cytoplasm which could directly inhibit protein synthesis. To study a differential damage to the nucleus and cytoplasm of an embryo and its effect on protein synthesis the experiments on haploid and ‘enucleated’ embryos were carried out. Diploid embryos were taken as a control.

After fertilizing the eggs irradiated at the dose of 20 kr with normal sperm androgenetic haploid embryos were obtained in which the father’s set of chromosomes was preserved, the mother’s one was inactivated and the cytoplasm was irradiated. While fertilizing normal eggs with the sperm irradiated at a dose of 40 kr gynogenetic haploid embryos were obtained, in which the father chromo-
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Fig. 3. Protein synthesis in loach embryos of various ploidy. At 6, 9, 12, 15 h after fertilization blastoderms were isolated from diploid gyno- and androgenetic haploid and 'enucleated' embryos of the loach, incubated with labelled amino acids for an hour, after that the specific activity of protein was determined. 1, Control; 2, gynogenetic haploids; 3, androgenetic haploids; 4, 'enucleated' embryos.

somes were inactivated, and the mother ones and the cytoplasm remained undamaged. Andro- and gynogenetic haploids developed with increasing abnormality up to hatching and then died at early larva stages (Neyfakh & Radziyevskaya, 1967).

After fertilizing irradiated eggs with irradiated sperm, 'enucleated' embryos virtually deprived of chromosomes were obtained. These eggs cleaved outwardly normally, reaching the blastula stages, although the rate of cleavage was slower than that of controls. After 13–15 h of development they perished. At the same period the control embryos reached the stage of mid-gastrula.

The dynamics of the incorporation of amino acids into protein in the above four series is shown on Fig. 3. The intensity of total protein synthesis in both haploid and diploid embryos differed little from one another. It increased drastically during gastrulation, though in androgenetic haploids the intensity of protein synthesis was usually somewhat lower than in gynogenetic ones. Protein synthesis in 'enucleated' embryos was much lower than in the control and shortly before the death (12 h after fertilization) reached approximately the level of 9 h control embryos at the late blastula stage. Consequently drastic inhibition
of protein synthesis due to the damage of nuclear structures was combined with the relatively insignificant direct effect of ionizing radiation on protein synthesis resulting from irradiation of cytoplasm. Thus the irradiation dose of 20 kr could be used while analysing the role of nuclei in the control of protein synthesis.

When fertilizing loach eggs with the sperm of goldfish diploid hybrids developing up to the larval stages were obtained, with foreign chromosomes functioning from early developmental stages. Androgenetic haploid hybrids, being a combination of goldfish chromosomes and loach cytoplasm, were incapable of differentiation, and their development was arrested at the stage of late blastula like that of 'enucleated' embryos of the loach. Thus goldfish chromosomes function in the cytoplasm in the presence of loach chromosomes, but they do not manifest themselves morphogenetically in the loach cytoplasm when the loach chromosomes are absent. The count of chromosomes showed that some of them were eliminated in diploid hybrids and in androgenetic haploid ones as well (Neyfakh & Radziyevskaya, 1967).
A biochemical study of some aspects of nuclear–cytoplasmic relations in the hybrid loach $\mathcal{F} \times $ goldfish $\mathcal{G}$ embryos was carried out. The data on the dynamics of protein synthesis obtained on intact embryos and isolated blastoderms showed that the intensity of protein synthesis was at the same level in diploid loach embryos and diploid hybrid loach $\mathcal{F} \times $ goldfish $\mathcal{G}$ embryos at the blastula and gastrula stages.

The dynamics of protein synthesis in haploid hybrids bears much resemblance to that of ‘enucleated’ embryos, i.e. no intensification of protein synthesis was observed, and 15–16 h after fertilization the level of the synthesis was nearly the same as in the control at the stage of late blastula (Fig. 4). On the contrary, synthesis of high-polymeric non-ribosomal RNA was much like that in the loach haploids and strongly differed from that in ‘enucleated’ embryos (Neyfakh, Timofeeva, Krigsgaber & Svetaylo, 1968). The rate of RNA synthesis in haploid hybrids was much higher than in the ‘enucleated’ embryos; although they did not significantly differ as to their early development, both groups of embryos were incapable of gastrulation and their development was arrested at the late blastula stage.

It follows from our data that the activation of the morphogenetic function of nuclei in diploid hybrids at blastula stages is followed by the activation of protein synthesis during gastrulation. However, the difference in protein synthesis between androgenetic haploid hybrid loach $\mathcal{F} \times $ goldfish $\mathcal{G}$ and androgenetic loach embryos, as well as the similar level of protein synthesis and the arrest of development in loach haploid hybrids and ‘enucleated’ embryos, suggest that RNA molecules synthesized on goldfish chromosomes were not involved in protein synthesis. The level of the latter was apparently provided by the templates stored in the loach cytoplasm during oogenesis.

Another means of chromosome ‘elimination’ was used to find out to what extent the arrest of development at the late blastula stage in androgenetic haploid hybrids was due to the elimination of goldfish chromosomes and to what degree it resulted from them being strange to the loach cytoplasm. For this purpose the chromosomes of loach male gametes in androgenetic haploids were partly eliminated by irradiation. Therefore loach eggs after their nuclei had been inactivated were inseminated with loach sperm previously irradiated in the dose of 1·75 kr. This dose was chosen because the death of the 50 % of such embryos took place 14–14·5 h after fertilization as in haploid hybrids, i.e. it was 2 h later than the death of the 50 % of ‘enucleated’ embryos. The dynamics of protein synthesis in such embryos did not virtually differ from androgenetic haploid hybrids loach $\mathcal{F} \times $ goldfish $\mathcal{G}$ (Fig. 4). RNA synthesis also proceeded similarly in both these types of embryos (Neyfakh, Timofeeva, Krigsgaber & Svetaylo 1968). These phenomena show that loach androgenetic haploid embryos with partly eliminated chromosomes can serve not only as a morphogenetic but also as a biochemical model of haploid hybrid embryos in which an intensive synthesis of high-polymeric RNA proceeds. Apparently a disagreement between the
goldfish chromosomes and loach cytoplasm first of all resulted in elimination of some chromosomes and in a 'distortion' of RNA synthesis without a reliable change in its quantitative characteristics. It again brings about inhibition of protein synthesis, arrest of development and death of embryos.

The data on the dynamics of protein synthesis in loach embryos irradiated or treated with actinomycin D 5–15 min after fertilization show that irradiation as well as chemical inactivation of the nuclei results in similar inhibition of protein synthesis (Fig. 5). Ionizing radiation applied in the developmental period of 0–5.5 h (early blastula) produced the same effect as irradiation or actinomycin D treatment of the eggs immediately after fertilization (Fig. 6A). In all the experimental series with the irradiated eggs the development was arrested at the late blastula stage, which was specific of the 'enucleated' embryos (Neyfakh, 1959). Protein synthesis was equally intense in the embryos in which the nuclei were inactivated at different moments of cleavage and early blastula. At 12–15 h after fertilization the intensity of protein synthesis corresponded to that of the control embryos at the late blastula stage (9 h of development). The experiments
of irradiation applied in early development and on ‘enucleated’ embryos and androgenetic haploid hybrids loach♀ × goldfish♂ indicated that up to the stage of mid-blastula the genetic apparatus of the cell was not virtually involved in protein synthesis. Hence it is possible to believe that up to the late blastula stage protein synthesis was programmed in oogenesis and was independent of the nuclei.

A totally different picture was observed when the inactivation of the nuclei was performed from 5·5 to 7·5 h of development (Fig. 6B). The later radiation was applied within this period the higher was the level of the specific activity of protein in irradiated embryos. While the development of the embryos irradiated 5·5 h after fertilization with the dose which totally blocked the nuclear function was arrested and they were at the late blastula stage by the level of protein synthesis (9 h of development), the inactivation of the nuclei performed 1 h later allowed the embryos to begin the gastrulation. Reaching the developmental stage of 12 h, they perished 15 h after fertilization. The activity of protein synthesis in these embryos approximately corresponded to that of 12 h control embryos.
Fig. 7. Protein synthesis in loach embryos irradiated at the stages of late blastula/early gastrula. Loach eggs were irradiated at a dose of 20 kr at 9, 11 and 13 h after fertilization. The intensity of $[^{14}C]$amino acid incorporation into blastoderm protein was studied during gastrulation and at the onset of organogenesis. 1, Control; 2, irradiation for 9 h, 3 for 11 h, 4 for 13 h after fertilization. The arrows indicate the moments of irradiation.

The synthesis was found to be more intensive in embryos irradiated 7.5 h after fertilization. These embryos lived longer and developed up to the stage of 15–16 h (mid-gastrula), the level of labelling being approximately the same as in 15–16 h control embryos. Consequently, at the stages of mid-blastula, protein synthesis was controlled by the nuclei and each hour of their functioning made protein synthesis more prolonged and intense. Evidently, the realization of the nuclear function during blastulation is required for the specific activation of protein synthesis during gastrulation. This period, which can be characterized as an active one with regard to the control over protein synthesis, coincided with the first manifestation of the morphogenetic function of nuclei (Neyfakh, 1959, 1964) and with an intensification of template activity of the genome (Kafiani, Timofeeva, Melnikova & Neyfakh, 1968; Kafiani, Timofeeva, Neyfakh, Melnikova & Rachkus, 1969). The partial elimination of chromosomes in loach androgenetic haploids also demonstrated the dependence of protein synthesis, in loach during gastrulation, on morphogenetic function of the nuclei and on the synthesis of RNA. The latter was capable of serving as a template for protein synthesis in vivo. Protein synthesis in androgenetic haploid loach embryos with partially
eliminated haploid chromosomes remained at the level of 'enucleated' embryos deprived of chromosomes, despite an intensive RNA synthesis. But this RNA seems not to realize its morphogenetic function either, for the development of the embryos was arrested at the late blastula stage while normal haploids of the loach pass all the stages of early development.

The study of the changes of protein synthesis of loach embryos irradiated at the late blastula mid-gastrula stages (9, 11, 13 h) gave similar results (Fig. 7). The activation of protein synthesis in such embryos proceeded for several hours and stopped 16–18 h after fertilization. But apparently the level of protein synthesis was enough for the completion of gastrulation.

The pattern of the curves showing the intensity of labelling after irradiation during mid-late gastrula (12, 15, 17 h of development) differed little from the control ones, which demonstrates the absence of a direct relationship with the nuclear function. At the same time protein synthesis proceeds rather intensively for a long time from the moment of the inactivation of the nuclei to the death of the embryos (Fig. 8). The curves expressing protein synthesis of the embryos with inactivated nuclei almost repeat the shape of the control curve. A similar picture was obtained on embryos irradiated at the developmental stages from 8 to 17 h.
It is known, however, that the so-called inactivation of the nuclear function induced by irradiation with high doses does not completely inhibit the synthesis of high-polymeric non-ribosome RNA (Belitsina, Gavrilova, Neyfakh & Spirin, 1963; Kafiani, Timofeeva, Neyfakh, Rachkus & Melnikova, 1966). While it is readily apparent from the data obtained on ‘enucleated’ embryos virtually deprived of chromosomes that protein synthesis is independent of transcription simultaneously proceeding at blastula stage, the independence of protein synthesis of simultaneous RNA synthesis during early differentiation is less obvious. The effect of irradiation and actinomycin D treatment on protein synthesis at different developmental stages was also studied on sea-urchin embryos, which are freely permeable for the solutions of inhibitors and other substances.

**Study of protein synthesis in sea-urchin embryos**

The rate of protein synthesis in normal development of Strongylocentrotus nudus embryos increased from the beginning of development up to the stage of mid-blastula, somewhat decreased during hatching and then increased again at the stages of mesenchyme blastula and gastrula. These results are in good agreement with the data on the change of protein synthesis obtained for early

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**Fig. 9.** Comparison of protein synthesis intensity in the sea urchin *S. nudus* early development after irradiation (A) and actinomycin (B) inactivation of the nuclei, at different times after fertilization.

(A) 2, 4, 6, 8 or 10 h after fertilization the eggs were irradiated at the dose of 15 kr. 10⁴ eggs were incubated in 1 ml of sea water with 0.5 μCi of [¹⁴C]lysine at each of successive developmental stages. The specific activity was expressed in cpm/mg of protein. (1), Control; (2), irradiation 2 h after fertilization; (3), 4 h; (4), 6 h; (5), 8 h; (6), 10 h.

(B) 2, 4, 6, 8 or 10 h after fertilization the eggs were placed into actinomycin solution (25 μg/ml) and protein synthesis intensity was determined. (1), Control; (2), the embryos placed into actinomycin 2 h after fertilization; (3), 4 h; (4), 6 h; (5), 8 h; (6), 10 h.
sea-urchin embryos of other species (Smith, 1963; Gross, Malkin & Moyer, 1964; Berg, 1965, 1968). Fig. 9 summarizes several experiments on protein synthesis in *S. nudus*. In all cases the specific activity of protein 6 h after fertilization (at the mid-blastula) at the end of the first period of the activation of protein synthesis was taken as 100% and the incorporation into protein at other stages of normal development was expressed as a percentage. The data on the intensity of protein synthesis after irradiation and actinomycin D treatment are also presented as a percentage of the control at corresponding developmental stages. The doses producing a complete effect were chosen. It means that the damage did not increase with the further increase of the dose. For X-rays it was a dose of 10–15 kr; for actinomycin D, 20 μg/ml. The data presented show that irradiation and actinomycin treatment of the eggs produce a similar effect on development and protein synthesis.

The difference between the effect of X-irradiation and actinomycin treatment is that the development of irradiated embryos was arrested but that they survived, while the actinomycin-treated embryos whose development was arrested at the same stages perished much earlier. It has been shown elsewhere that irradiation does not block mRNA synthesis but inhibits its increase in the course of development (Kafiani et al. 1966). At the same time actinomycin D almost completely inhibited mRNA synthesis. Evidently the high level of RNA synthesis into RNA does not reflect the ability of this rapidly labelled RNA to serve as a template for protein synthesis. A similar conclusion was drawn from the studies on mRNA and protein synthesis in haploid androgenetic hybrids loach ♂×goldfish ♀.

The inactivation of the nuclei in irradiated or actinomycin-treated sea-urchin embryos in the period from fertilization to the stage of 32–64 blastomeres produced no appreciable effect on protein synthesis up to the early blastula stage. However, the rate of protein synthesis decreased more abruptly in experimental embryos 5–7 h after fertilization than in the control. The development of such embryos had been arrested before they reached the stage of hatching. Both factors applied at early blastula stage inhibited protein synthesis, which manifested itself virtually at once. A decrease of labelling in embryos irradiated or treated with actinomycin at the stages of early/mid-blastula (4–6 h after fertilization) was more intensive at the stages of hatching and mesenchyme blastula and prevented the onset of gastrulation. Evidently the nuclear function at the early blastula, when high polymer non-ribosomal RNA was actively synthesized by the nuclei (Timofeeva, Ivanchik & Neyfakh, 1969) provided for the activation of protein synthesis at the stages of mesenchyme blastula. On the contrary, when X-rays and actinomycin D were applied during hatching and passage to the mesenchyme blastula, protein synthesis and development were considerably less affected. Actually such embryos morphologically did not differ from the control. Protein synthesis was also nearer to the control. Protein synthesis in these embryos was activated during mesenchyme blastula stage,
i.e. simultaneously with the control embryos. The pattern of protein synthesis after actinomycin treatment resembled that in the control embryos though on a lower level.

**DISCUSSION**

Three aspects of the data obtained can be considered. The first one is concerned with the relationship between nuclear morphogenetic function and nuclear control over protein synthesis. This relationship is expressed by the fact that the intensity of protein synthesis up to the early blastula in the sea urchin and to the late blastula stage in the loach is independent of the embryo’s nuclei, when their morphogenetic function does not manifest itself. However, at this early developmental period when protein synthesis and development are independent of the embryo’s genome, the morphogenetic function of the nuclei providing early differentiation occurs. The inactivation of the nuclei at the early blastula in the sea urchin and mid-blastula stage in the loach allows the dependence of protein synthesis on nuclear control to be revealed. One can see that the morphogenetic nuclear function provides a specific activation of protein synthesis concerned with mesenchyme blastula in the sea urchin and gastrulation in the loach. Finally, at the stages of hatching and mesenchyme blastula in the sea urchin and gastrula in the loach, when the morphogenetic function of the nuclei is weaker, the nuclear control over protein synthesis is also less pronounced. This time the coincidence between the morphogenetic activity of the nuclei and nuclear control over protein synthesis is supposed to be due to the fact that the control over protein synthesis is the first manifestation of the nuclear morphogenetic function. It is but natural that the nuclear control over protein synthesis is realized more directly than over morphogenetic processes.

The second problem to be discussed is concerned with the relationships between synthesis of mRNA and protein. The difference between normal intensity of protein synthesis in the control embryos and protein synthesis in the embryos after the inactivation of the nuclei is determined by blocking the synthesis of new mRNA, breakdown of already synthesized short-living mRNA, and by the changes of protein synthesis related to non-gene mechanisms of regulation. We have shown that if the activity of genome is suppressed, protein synthesis is inhibited from the stage early/mid-blastula in the sea urchin and late blastula in the loach. It seems to be due to the fact that the supply of mRNA stored in the egg is virtually utilized by these developmental stages (Glisin, Glisin & Doty, 1966; Rachkus, Kuprianova, Timofeeva & Kafiani, 1969). The intensification of protein synthesis during early differentiation which begins after that proceeds only when the mRNA synthesis occurs at early blastula stages in the sea urchin and mid-blastula in the loach. Although the labelling of mRNA is revealed at the stages of 2-4 blastomeres in the sea urchin, the activation of transcription takes place at early blastula stage (Timofeeva et al. 1969). Similar activation of transcription in the loach proceeds at mid-blastula stage
Nuclear control of protein synthesis (Kafiani et al. 1969). The dependence of intensification of protein synthesis at the stage of mesenchyme blastula in the sea urchin and gastrula in the loach on the nuclear function at early blastula in the sea urchin and mid-blastula in the loach seems to be caused by the activation of transcription at the developmental stages indicated.

At the same time the dependence of protein synthesis on synthesis of high-polymeric non-ribosomal RNA is not so simple. In gynogenetic haploid loach embryos the intensity of protein synthesis is the same as in diploid ones, although the intensity of RNA synthesis is about two times lower (Kafiani et al. 1968). On the contrary, protein synthesis in haploid hybrid loach $\varphi \times$ goldfish $\sigma$ embryos remains at the level of 'enucleated' embryos although RNA synthesis is activated and proceeds with the same rate as in loach haploids (Neyfakh et al. 1968). Hence it can be suggested that not only is the intensity of RNA transcription of importance but also the ability of newly synthesized RNA to be a template for protein synthesis.

The role of 'non-gene' mechanisms in the control over protein synthesis is the third aspect of interest. Their role can be revealed when the genetic control is blocked. Our results on the independence of the activation of protein synthesis in the sea urchin after fertilization when the nuclear activity is suppressed agree with the data on the absence of direct genetic control over protein synthesis at these early developmental stages (Gross & Cousineau, 1963; Denny & Tyler, 1964). A decrease of protein synthesis during hatching in the sea urchin when mRNA synthesis is most active and the activation of protein synthesis at the stages of mesenchyme blastula which precedes when transcription is blocked with actinomycin seems to be controlled at polysome level (Infante & Nemer, 1967; Terman, 1970).

It is shown in the experiments on loach 'enucleated' embryos and after the inactivation of nuclei at the stages from fertilization to mid-blastula that duration and intensity of protein synthesis in all these experimental versions is the same and protein synthesis reaches the level characteristic of control embryos at the late blastula stage. Protein synthesis in the loach up to the onset of gastrulation seems to proceed on the templates stored in oogenesis. In spite of the nuclei having been inactivated at the stages of late blastula/mid-gastrula the inactivation of protein synthesis in the loach during gastrulation takes place. It seems to be due to the fact that active polysome complexes are formed at the gastrula stages with the participation of mRNA which have been synthesized as early as the blastula stages, while a good deal of templates involved in synthesizing machinery is formed at the expense of informosome degradation (Belitsina, Aitkhozhin, Gavrilova & Spirin, 1964; Spirin, Belitsina & Aitkhozhin, 1964).

Hence protein synthesis during cleavage and blastulation (sea urchin, loach) at the mesenchyme blastula (sea urchin) and during gastrulation (loach) also turns to be programmed by the transcription of the genome during preceding developmental stages such as oogenesis and early (sea urchin) and mid-blastula (loach)
stages. Thus a time gap between the occurrence (mRNA synthesis) and the first manifestation (protein synthesis) of morphogenetic function of nuclei is observed. It is provided by long-living mRNA whose template activity is temporarily inhibited (informosomes, inactive polysome complexes), and by ‘non-gene’ mechanisms which directly regulate the changes in the intensity of translation. These and some other mechanisms provide a relative independence of protein synthesis of the simultaneous mRNA synthesis as well as its dependence on the activity of the nuclei at preceding developmental stages.

The authors wish to thank Professor G. V. Lopashov for his helpful suggestions and criticism in the course of preparation of the manuscript for publication.

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(Manuscript received 23 December 1971, revised 13 March 1972)