

Alterations in the morphology of nuages in spermatogonia of the fish, *Oryzias latipes*, treated with puromycin or actinomycin D

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Summary — A germ-cell specific organelle, nuage, in *Oryzias latipes* is polymorphic. Nuages with a strand-like structure and amorphous fibrous bodies can be discriminated from each other; furthermore 2 types of nuages of intermediate morphology are also present. After the administration of puromycin or actinomycin D, nuage morphology in spermatogonia was examined by electron microscopy. Puromycin as well as actinomycin D caused a significant increase in the incidence of nuages with a strand-like structure. These observations indicate that the loss of supply of some materials can induce morphological changes in nuages, suggesting that the polymorphism in nuages of *O. latipes* possibly results from the turnover of nuage materials.

***Oryzias latipes* / teleost / nuage / spermatogonium / ultrastructure**

Résumé — **Altération de la morphologie des nuages dans les spermatogonies du poisson, *Oryzias latipes*, traité à la puromycine ou l'actinomycine D.** *Un organite spécifique des cellules germinales, le nuage, est polymorphe chez *Oryzias latipes*. Des nuages filamenteux et des corps fibreux amorphes peuvent être reconnus; 2 types de nuages de morphologie intermédiaire existent en outre. Après l'administration de puromycine ou d'actinomycine D, la morphologie des nuages dans les spermatogonies a été observée au microscope électronique. Ces inhibiteurs provoquent une augmentation significative de la fréquence des nuages filamenteux. Ces observations indiquent que l'arrêt de fourniture de matériaux peut induire des modifications morphologiques des nuages et suggèrent que le polymorphisme des nuages chez *O. latipes* résulte d'un renouvellement des matériaux du nuage.*

***Oryzias latipes* / téléostéen / nuage / spermatogonie / ultrastructure**

INTRODUCTION

The electron-dense materials which are referred to by various names (nuages, nucleolus-like bodies, intermitochondrial cement, chromatoid bodies, germinal dense bodies, electron-dense substances, and so on) exist in germ cells of many organisms (Eddy, 1975). Changes in their morphology during development of germ cells have been noted in amphibians and insects (Mahowald, 1968, 1971, 1975; Kalt, 1973; Ikenishi and Kotani, 1975), but the function of these germ-cell specific materials has not been clarified so far.

In previous studies (Hamaguchi, 1982, 1985, 1987), we indicated that the germinal dense bodies (nuages) in the teleost, *Oryzias latipes*, were polymorphic; nuages with a loosely woven strand-like structure and amorphous fibrous bodies could be discriminated from one another. Two types of nuages with intermediate structures were also present. These 4 types of nuages co-existed in germ cells and their incidence varied according to the progress in developmental stages of germ cells. Primordial germ cells of *Oryzias latipes* in the endoderm contained nuages only with a strand-like structure. During migration to the gonadal anlage, strand-type nuages decreased and the amorphous-type increased instead (Hamaguchi, 1985).

In female germ cells, amorphous nuages continue to increase and > 90% of nuages in oocytes at the diplotene stage are amorphous in type (unpublished data). On the other hand, in the male germ line, the proportion of strand-type nuages increased (Hamaguchi, 1987). This difference in nuage structure between oogonia and spermatogonia is the first indication of the sex differentiation of *O. latipes* germ cells at the ultrastructural level (Hamaguchi, 1982). However, the mechanisms of these changes in nuage structure has not

been clarified; the biological significance of their polymorphism is therefore also unknown.

Based on the incorporation of radioactive amino acids, Eddy and Ito (1971) reported a rapid turnover of the materials in the dense bodies of amphibian germ cells. In the previous paper (Hamaguchi, 1985), we discussed the possibility that nuage polymorphism in *O. latipes* was related to the synthetic activity of the nuage materials. In order to verify this hypothesis, we examined the effects of protein synthesis and of RNA synthesis inhibitors on nuage morphology.

MATERIALS AND METHODS

The *d-rR* strain of the teleost, *Oryzias latipes* was used in this study. For the treatment with inhibitors, testes were dissected out and incubated at $25 \pm 0.5^\circ\text{C}$ *in vitro* in tissue culture medium 199 containing 100 units penicillin and 100 μg streptomycin/ml medium. Puromycin (20 μg /ml) (Makor Chemical Ltd, Jerusalem, Israel) and actinomycin D (20 μg /ml) (PL Biochemicals Inc, Milwaukee, USA) were added to this culture medium. Tissues were incubated in Pyrex disposable culture tubes containing 0.25–0.50 ml culture medium, and gently shaken during the incubation period. After 24, 36 or 48 h of incubation, testes were routinely fixed in a formaldehyde–glutaraldehyde–picric acid combination fixative (Ito and Karnovsky, 1968), post-fixed in OsO_4 , dehydrated in ethanol and embedded in epoxy resin. Thin sections were cut, stained with uranyl acetate and lead citrate, and examined under an H-300 electron microscope (Hitachi, Japan).

RESULTS

As indicated in the previous paper (Hamaguchi, 1987), nuages in type A spermatogonia were polymorphic, and various types of nuages co-existed in a spermatogonium (fig 1). The type of each nuage

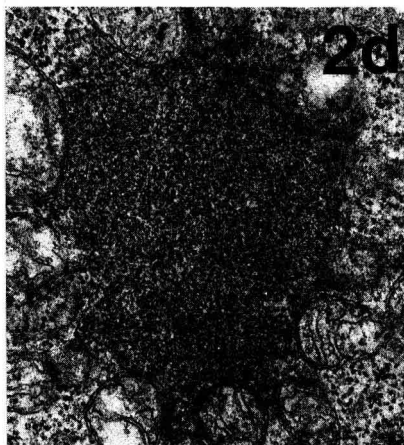
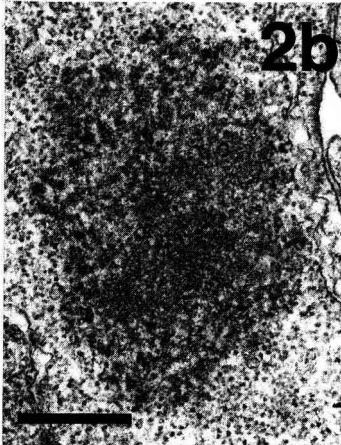
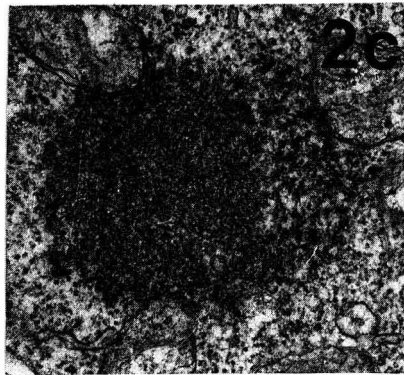
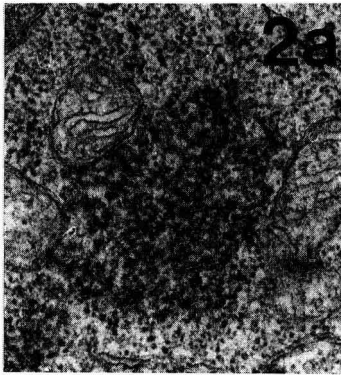
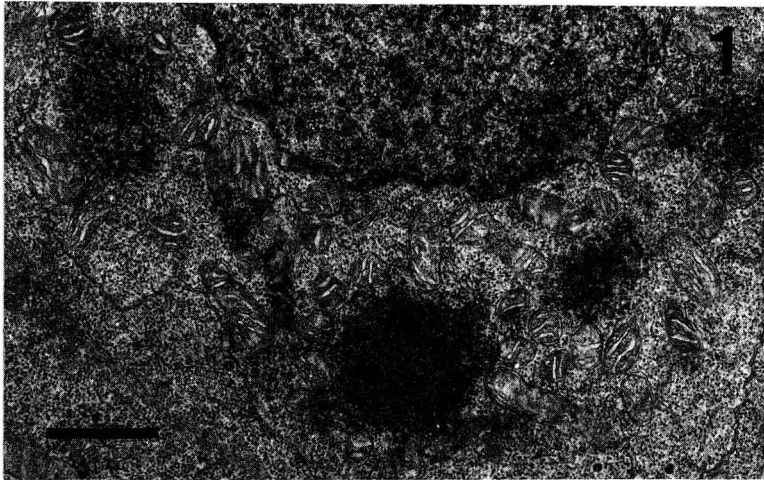


Fig 1. Spermatogonium in a testis of control group after 48 h incubation. Nuages of various types can be seen. Bar: 1 μm .

Fig 2. Four types of nuages. a: Strand-like structure; b: intermediate strand-like structure with a small amorphous mat of fine dense fibrils; c: intermediate structure in which the amorphous mat is dominant; d: amorphous body of fine electron-dense fibrils. Bar: 0.5 μm .

was determined according to the classification introduced in the previous study (Hamaguchi, 1985, 1987), in which 4 types of nuages were discriminated: type A, nuages with a strand-like structure; type B, nuages with an intermediate structure in which the strand-like structure is dominant; type C, nuages with an intermediate structure in which the amorphous mat portion is dominant; type D, amorphous fibrous bodies (fig 2a–d). From observations on > 100 nuages, incidences of 4 types of nuages were calculated. Numbers of testes, cells and nuages examined are indicated in figure 2.

The results are shown in figure 3. The morphology of nuages in type A spermatogonia did not significantly change over the 48-h incubation period under the present study conditions. Drug administration did not induce abnormally structured nuages, and the morphology of all nuages observed could be determined according to the classification indicated above.

When puromycin was added, the morphology of nuages changed significantly; nuages with a strand-like structure increased after 24 h incubation. In testes treated with puromycin, 74% of nuages were type A or B at 24 h, 69% at 36 h and 82% at 48 h; whereas, in control testes, there were type A or B nuages at 24, 36 and 48 h, 46, 48, 56% respectively. Differences could also be noted between the actinomycin D-treated group and the control. Nuages with a strand-like structure increased after actinomycin D administration. However, actinomycin D at the present concentration was less effective in altering nuage morphology than puromycin.

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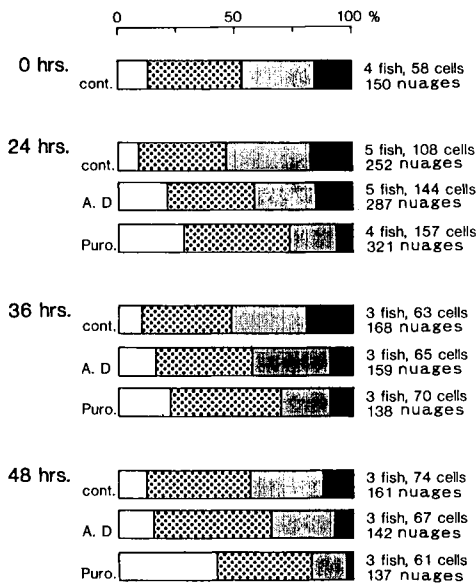


Fig 3. The incidences of 4 types of nuages in the spermatogonia after incubation with or without inhibitors. The columns are divided according to the percentages of each type of nuage. The numbers of specimens, cells and nuages observed are shown. □: type A; ▨: type B; ▩: type C; ■: type D nuages.

DISCUSSION

The present investigation revealed that the inhibitors of protein or RNA synthesis can change nuage morphology in spermatogonia. There was a relative increase in nuages with a strand-like structure and an inversely proportional decrease in amorphous fibrous bodies. Kalt *et al* (1975) and Söderström (1977) have also reported that inhibitors can induce alterations in the morphology of chromatoid bodies of *Xenopus* and rat, which are known to be electron-dense cytoplasmic inclusions specific to male germ cells. However, according to the report by Söderström (1981), chromatoid bodies in the spermatocytes or spermatids are separate organelles from nuages in spermatogonia; therefore, the

present results provide the first information that the synthetic activities are related to the polymorphic structure of nuages.

Using radioactive tracers, Eddy and Ito (1971) reported a rapid turnover of the proteinaceous materials in amphibian nuages. Azevedo (1984) has shown that radioactive uridine can be incorporated into the nuages in the oocytes of a viviparous teleost. Our preliminary results (unpublished data) also indicate that nuages in spermatogonia as well as oogonia and oocytes can incorporate radioactive phenylalanine and uridine within 30 min of intraperitoneal injection. These facts support the notion that nuages are not stable organelles, but that the materials which constitute nuages are under active turnover. The present results indicate that the alteration in the synthetic activity of the germ cells can induce the morphological changes in nuages, and that nuage polymorphism is possibly a reflection of synthetic activities of nuage materials.

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