Studies on the Histones of the Ciliate *Stylonychia mytilus*

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Abstract. Histones were extracted from pure macronuclei and macronuclear anlagen and were analyzed by different electrophoretic techniques and by amino acid analysis. Fractionation of the histones on SDS-gels showed that the histone fractions of the macronucleus and the macronuclear anlagen are identical in their molecular weights. Comparison with calf thymus histone fractions showed considerable similarities in the molecular weights. Analysis by polyacrylamide-urea gel electrophoresis and by amino acid analysis showed quantitative differences in some histone fractions between these two types of nuclei.

Introduction

It has been suggested that histones, the basic proteins of the eukaryotic nucleus, may be involved in gene regulation (Hnilica, 1972), but the exact nature of this regulation is still very unclear. In several species it has been shown that the histone pattern changes during embryonic development (Fambrough *et al.*, 1968; Asao, 1969; Evans and Ozaki, 1973; Tessier and Pallotta, 1973) and the demonstration that the histone composition influences the fine structure of the chromatin (Allfrey and Mirsky, 1963; Pallotta *et al.*, 1970) suggests that histones might play a role in the control of gene activity.

The nuclear dualism of ciliates with a metabolically active macronucleus and one or more inactive generative micronuclei provides a good system for the comparison of different types of nuclei in one cell. Moreover, the development of the new macronucleus after sexual reproduction allows the study of possible changes in histone pattern during this developmental process.

In the hypotrichous ciliate *Stylonychia mytilus* the macronucleus breaks down during conjugation, the micronuclei undergo meiosis to form haploid gametic nuclei which are exchanged between the partner cells and then form a diploid syncaryon. After mitotic division of the synkaryon one of the daughter nuclei develops into a new macronucleus whereas the other differentiates into micronuclei. In the macronuclear anlage an increase in the DNA content takes place and polytene giant chromosomes are formed (Ammermann, 1968). These giant chromosomes disintegrate and more than 90% of the DNA is lost from
the nucleus. The anlage elongates and reaches its final DNA content by progressive doubling of the quantity of DNA (Ammermann, 1971). Whereas the vegetative macronucleus synthesizes RNA during the whole cell cycle, no RNA is synthesized in the macronuclear anlage (Ammermann, 1970). Also the DNA of the macronuclear anlage and of the micronuclei is enriched in repetitive sequences which are not present in the vegetative macronucleus (Ammermann et al., 1974).

It has already been shown that there are differences in histone pattern between the macronucleus and the macronuclear anlage in the giant chromosome stage (Lipps et al., 1974), so it seemed to be of interest to investigate these differences in more detail by means of different electrophoretic techniques and by amino acid analysis of the histones from different developmental stages.

**Material and Methods**

Normal cells and emicronucleate cells (Ammermann, 1970) of *Stylonychia mytilus*, syngen I (Ammermann, 1965) were grown in neutral Pringsheim solution (Ammermann et al., 1974) and were fed daily with the flagellate *Chlorogonium elongatum*. One to two days before isolation of nuclei feeding was stopped. The cells were concentrated, lysed and the nuclei were collected and purified from other cytoplasmic particles as previously described (Lipps et al., 1974). The purification of macronuclei from micronuclei was accomplished by filtration through nylon gauze (Lipps et al., 1974) or by using emicronucleated stocks. Isolation of the macronuclear anlagen in the giant chromosome stage was performed according to the technique described by Ammermann et al. (1974).

Histones were extracted with 0.25 N HCl from whole nuclei or from purified chromatin. Extraction from whole nuclei was accomplished by mechanical homogenization in dilute HCl. Chromatin was isolated by extraction of nucleohistones with 2 M NaCl pH = 3.6 and by dilution to a final salt concentration of 0.15 M NaCl (Butler, 1964). From all these preparations the acid soluble supernatant was precipitated with 10 volumes of acetone for 24 hours at −20 °C. The precipitates were then washed three times in acid acetone, three times in acetone and then dried in vacuo.

Fractionation of histones was carried out by technique 1 of Johns (1964) for the fractions F1, F3, F2A and F2B. Fractionation of F2A into F2A1 and F2A2 was performed according to method 1 of Johns (1967). Starting from about 80 liters of culture (about 5 × 10⁷ cells), the yield of the different histone fractions was between 1 and 2 mgs. Whole calf thymus histones and calf thymus histone fractions were isolated in similar manner as those from *Stylonychia* nuclei. To avoid degradation of histones during the extraction procedure, all preparations were made in the presence of either 0.05 M sodium bisulfite or 1 mM diisopropylfluorophosphate (Isaacks and Santos, 1973).

Electrophoresis of histones was performed either on polyacrylamide-urea gels or on polyacrylamide-SDS gels. Gel dimensions were 0.5 cm × 8.5 cm or 0.5 cm × 12.5 cm.

Electrophoresis on polyacrylamide-urea gels was performed on 15% acrylamide gels containing 2.5 M or 6.25 M urea and 0.9 N acetic acid according to the technique described by Panyim and Chalkley (1969a) or on 15% acrylamide gels