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Modulation of hepatic chromatin structure in response to 17-β estradiol induced activation of the vitellogenin gene regions in Atlantic salmon, *Salmo salar**

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Only small fractions of the eukaryotic genome are utilized for gene expression at any one time. Most of the non-expressed genetic information is packaged within the interphase nucleus into chromatin fibers. These fibers consist of a higher order coil of a repeating subunit of chromatin, the nucleosome. Each nucleosome consists of a histone octamer, 2 each of H2A, H2B, H3 and H4. Histone Hl may associate with the nucleosome fiber [1]. A number of models on the structural configuration of the fibers have been presented (review: Felsenfeld & McGhee [2]). Cations are essential for the condensation and for keeping the chromatin in the solenoid structure. The cations bind to the chromatin and neutralize the negative charges of the DNA backbone, thus lessening the repulsive forces in the chromatin fiber [3]. The higher order structure is facilitated, integrated and modified by a number of DNA-binding proteins as well as positively charged components [4, 5].

A heterogeneous group of non-histone proteins is present in chromatin. This group consists of structural proteins, enzymes and gene regulators. Fractionation of chromatin and electrophoretic separation combined with immunoblotting or two-dimensional electrophoresis are required to obtain a distinct resolution of the non-histone proteins [6, 7]. The high mobility group (HMG)[1] proteins are a subgroup of well characterized non-histone proteins. An association with transcriptionally active chromatin has been suggested [8, 9]. In rainbow trout (*Oncorhynchus mykiss*) the proteins HMG Tl and HMG T2 are similar to mammalian and avian HMG 1 and HMG 2 [10] and three proteins D, C and H6 are similar to mammalian and avian HMG 14 and HMG 17 [11].

Polyamines are among the components of positive charge and, in addition to the histones, bring about a neutralization of chromatin phosphates [12]. Polyamines are involved in various cellular processes acting as cofactors to enzymes. The main effect of spermine and spermidine is to participate in or to stabilize the condensation of the chromatin fibers by electrostatic or physico-chemical interactions [12].

The regulation by estrogen of yolk protein synthesis in liver of oviparous vertebrates is a well defined process [13]. Males and juveniles can be induced experimentally and represent a true primary reaction to the hormone. Atlantic salmon (*Salmo salar*) can be induced by 17-β estradiol to synthesize vitellogenin in liver [14]. Large scale alterations in the nuclei are required to make possible the synthesis of vitellogenin [4, 15]. A change in the amount of chromatin proteins and a shift in their distribution between condensed and dispersed chromatin may be responsible for the accessibility of genes to transcription into RNA. Micrococcal nuclease (MNase) preferentially digests transcriptionally active chromatin into nucleosomes [16 - 18]. Following hormone treatment of juvenile Atlantic salmon, less chromatin was

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[1]Abbreviations: HMG, high mobility group proteins; MNase, micrococcal nuclease; S-fraction, soluble fraction

digested by the endonuclease and recovered in the soluble fraction (S-fraction) as compared with the non-hormone treated controls (Fig. 1) [5, 15].

Hybridization experiments with a cDNA probe specific for the vitellogenin gene revealed the release of the vitellogenin gene into the S-fraction after a brief exposure of the chromatin to MNase (Fig. 2) [5, 15]. Although large domains of the chromatin became condensed by the treatment with 17-β estradiol the highly transcribed vitellogenin gene was present in a decondensed configuration.

A large number of proteins were associated with the soluble chromatin [4]. Among the proteins that increased in content, a 21 kDa protein was most prominent (Fig. 3) [15]. The increase was noted in the total chromatin and more so in the S-fraction, which contains the chromatin solubilized by MNase (Fig. 3).

The electrophoretic mobility of the 21 kDa protein was similar to that of the rainbow trout HMG D protein. Following MNase treatment the protein was found in the mononucleosomal fraction, indicating an association with transcriptionally active chromatin. Isoelectric focusing separated the protein into 5 distinct spots which upon SDS-polyacrylamide gel electrophoresis showed identical mobility (Fig. 4) [19].

Upon trypsin digestion of the 5 electrophoretic fractions, peptides of the same electrophoretic mobility in SDS-polyacrylamide gels were obtained [19]. The separation of a single protein into several spots by isoelectric focusing indicated a secondary modification, possibly by acetylation as has been reported for histones [20]. The N-terminal amino-acid sequence of the 21 kDa protein showed 95% similarity with that of the HMG D of rainbow trout (Fig. 5). The amino-acid composition also coincided with that of the HMG D of rainbow trout [11, 21]. From these results it has been concluded that the 21 kDa protein belongs to the family of HMG proteins. The conserved regions of the N-terminal amino-acid sequence indicated a relationship of HMG protein families between the mammals, avians and teleost fishes. It is most likely that the relationship extends to other species along the evolutionary pathway.

Treatment of Atlantic salmon with 17-β estradiol caused a proliferation of the liver with an increase in wet weight and unchanged concentration of DNA/g wet weight of liver (Table 1) [26]. Polyamines are associated with prolif-

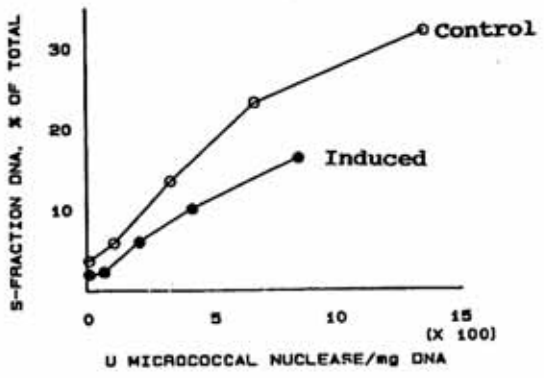

Fig. 1. Chromatin released by micrococcal nuclease. Nuclei were incubated with increasing amounts of micrococcal nuclease/mg of DNA. The chromatin was fractionated into a 102000 × *g* supernatant fraction (S-fraction) and pellet. The DNA was measured as absorbance at 260 nm and the released DNA expressed as percentage of the total DNA. ○, Control fish; ●, 17-β estradiol treated fish. Modified from Waters & von der Decken [15]

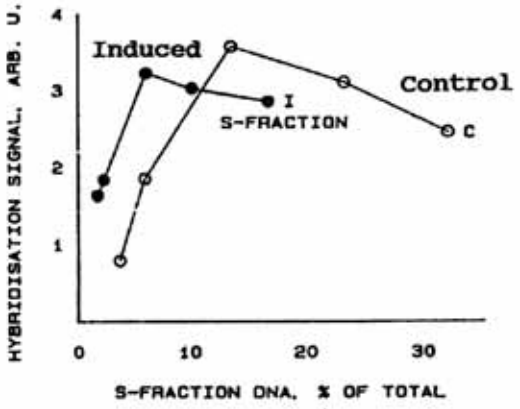

Fig. 2. DNA-cDNA hybridization of the vitellogenin gene.
Nuclei were incubated with increasing amounts of micrococcal nuclease/mg of DNA. The chromatin was fractionated into a 102000 × *g* supernatant (S-fraction) and pellet. The S-fraction was hybridized with vitellogenin specific cDNA labelled with tritium to 7×10^7 d.p.m./μg DNA. The extent of hybridization is expressed as arbitrary units. Modified from Waters & von der Decken [15]

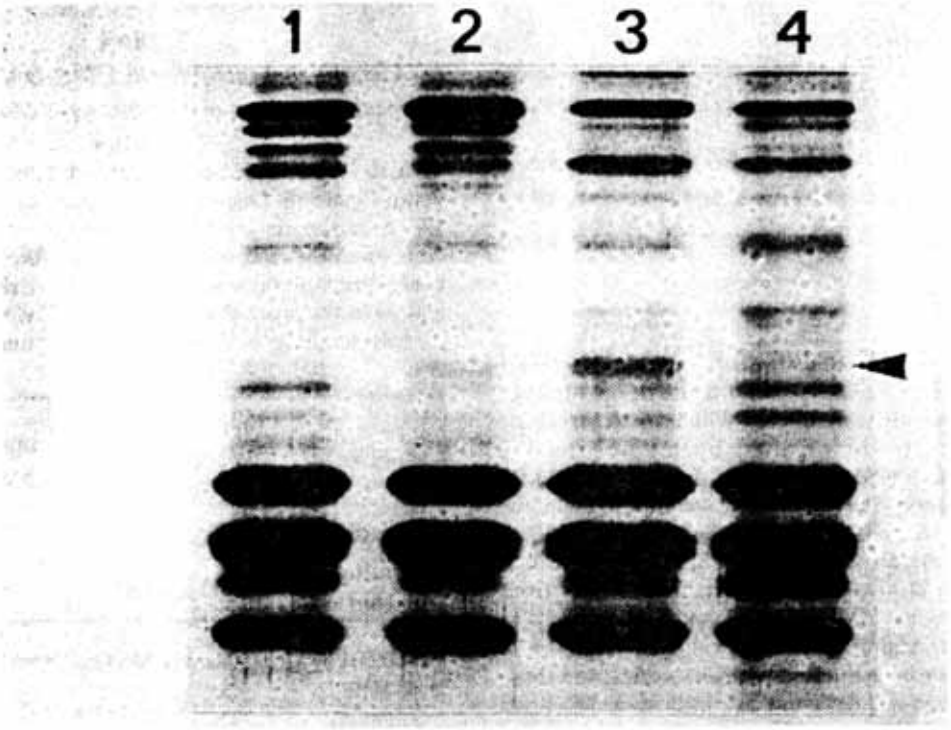

Fig. 3. SDS-polyacrylamide gel electrophoresis of the total nuclear proteins and the S-fraction.
Nuclei were incubated with micrococcal nuclease under such conditions that 5% of the chromatin was released into the S-fraction. The total nuclear fraction (Lanes 1 and 2) and the S-fraction (Lanes 3 and 4) were electrophoresed. Lanes 1 and 4, control fish; lanes 2 and 3, 17-β estradiol treated fish. The amount of protein applied to each lane was 5 μg. The arrowhead indicates the 21 kDa protein. The major proteins of a size below the 21 kDa are the core histones. The proteins of a molecular mass below 35 kDa are shown in the Figure. Modified from Waters & von der Decken [15]

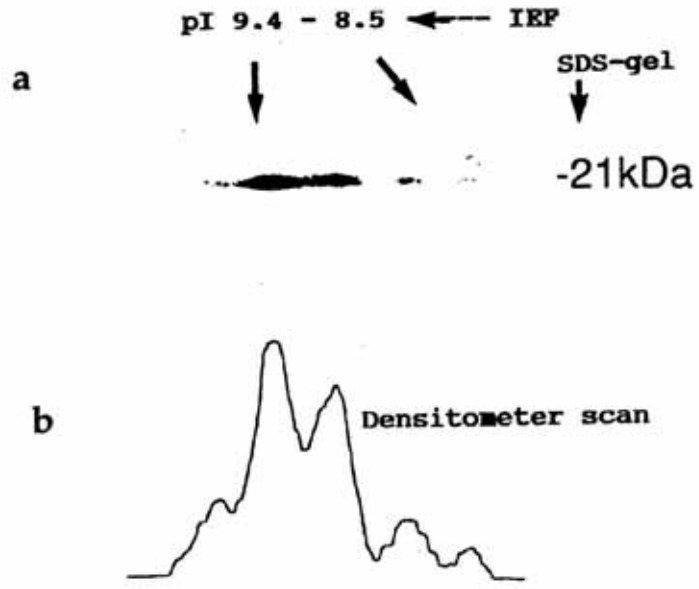

Fig. 4. Two dimensional electrophoresis of the 21 kDa protein.
Hepatic nuclei were extracted with 0.75 M perchloric acid. The extracted proteins were fractionated by h.p.l.c. The peak analyzed by SDS-polyacrylamide gel electrophoresis as the 21 kDa protein was subjected to isoelectric focusing in one dimension followed by SDS-polyacrylamide gel electrophoresis in the second dimension. The protein resolved into 5 distinct spots after isoelectric focusing. (a) The gel was stained with Coomassie Brilliant Blue. (b) Densitometric scanning of the stained gel. Modified from Waters *et al.* [19]

21 kDa protein *Salmo salar*		PKRKQ GAAGD DKEEP QRRR
HMG D	Trout	***** ***** V**** ****
HMG H6	Trout	****S ATK** EPARR SA*L
HMG 14A	Chicken	****A P*E*E A**** K**S
HMG 14	Human	****V SS*EG AAK*E PR**

Fig. 5. Amino acid sequence of the N-terminal residues of the 21 kDa protein and HMG proteins.
The nuclear proteins were treated with 0.75 M perchloric acid, the extract was purified by h.p.l.c. and the N-terminal amino acid sequence of the isolated 21 kDa protein determined. The sequence was compared with the sequences in the Swiss-Prot Data Bank by the FASTA program of Pearson & Lipman [22]. The N-terminal sequence of the 21 kDa protein, trout HMG D [11], trout HMG 6 [23], chicken HMG 14A [24], and human HMG 14 [25] are shown. Amino acids identical to those in the 21 kDa protein have been marked with an asterisk, and those amino acids that are different are indicated. Modified from Waters *et al.* [19]

Table 1

Hepatic wet weight and DNA content after a period of 14 days of continuous exposure to 17-β estradiol

Silastic capsules were implanted into each fish by a ventral incision. One group received capsules filled with 17-β estradiol, the control group empty capsules. The fish were anaesthetized with 3-aminobenzoic acid ethyl ester before implantation. After 2 weeks the fish were killed by a blow to the head, the liver removed, weighed and used for further analysis. The results are the means ± SEM

Fish	Liver wet weight g/100 g body weight	mg DNA/g wet weight of liver
Control	0.75 ± 0.02*	2.14 ± 0.04
Estradiol -treated	1.03 ± 0.06*	2.16 ± 0.12

*$P < 0.001$ by Student's *t*-test. Modified from Waters & von der Decken [26]

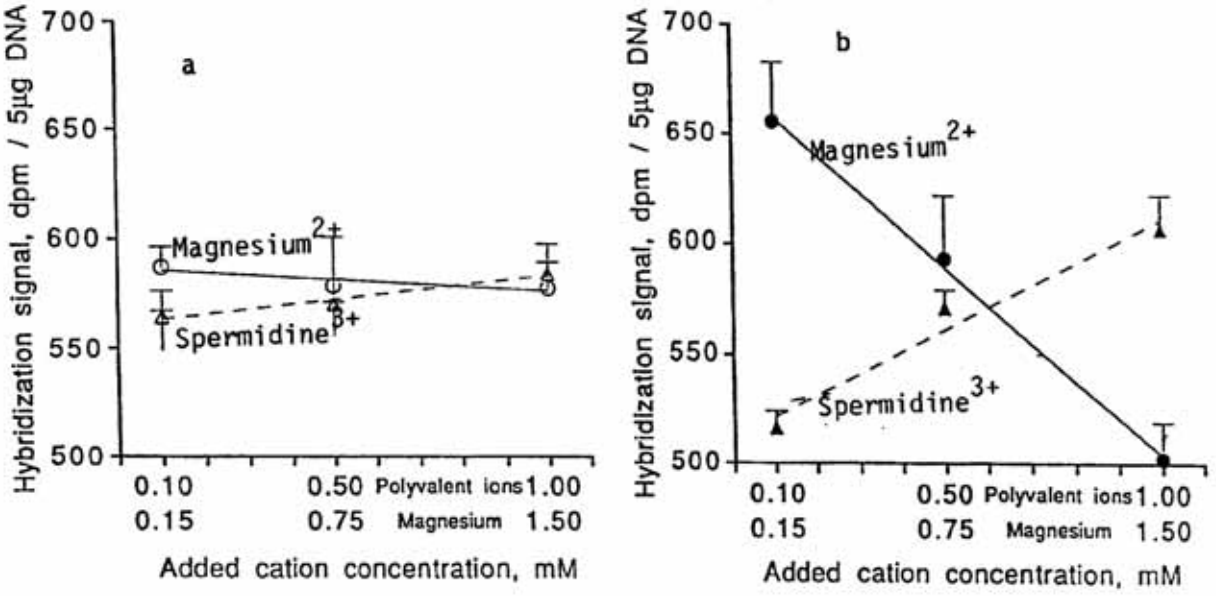

Fig. 6. Hybridization of vitellogenin specific [^{3}H]cDNA to DNA isolated from the condensed chromatin of the pellet fraction after incubation of nuclei with the restriction enzyme EcoRI and magnesium^{2+} or spermidine^{3+}.
Nuclei were incubated with *Eco*RI and the chromatin separated into a supernatant and pellet fraction. The DNA of the pellet fraction was isolated after incubation with proteinase K followed by phenol extraction and precipitation with ethanol. An amount of 5 µg DNA was used for hybridization. (a) Control fish. The slopes of the lines were not significantly different from zero. (b) Fish treated with 17-β estradiol. The slopes differed significantly from zero ($P < 0.05$) and from each other ($P < 0.025$). The background obtained with calf thymus DNA (117 ± 2 d.p.m.) has been subtracted. Modified from Waters & von der Decken [29]

Table 2

Concentration of polyamines in liver and muscle after treatment of Atlantic salmon with 17-β estradiol

Polyamines were extracted from liver and muscle and further purified after addition of the internal standard n-butylamine. The polyamines were applied to Titan III cellulose acetate strips. After electrophoresis the strips were developed using *o*-phthaldialdehyde and the polyamines detected by fluorescence with excitation at 366 nm. The plates were photographed and the negatives of the film analyzed in a Molecular Dynamics Densitometer. The curves were computed to nmol polyamines/g wet weight of tissue. The results are the means ± SEM

Fish	Tissue	Polyamines, nmol/g wet weight		
		Putrescine	Spermidine	Spermine
Control	Liver	365 ± 30*	382 ± 32*	334 ± 34
Estradiol-treated		1125 ± 88*	748 ± 71*	345 ± 33
Control	Muscle	15 ± 2.3	16 ± 3.5	27 ± 5.2
Estradiol-treated		21 ± 2.7	27 ± 9.3	31 ± 6.2

*$P < 0.001$ by Student's *t*-test. Modified from Waters *et al.* [5]

erating tissues and their objective is many-sided [27]. After treatment of the fish with 17-β estradiol, putrescine and spermidine, but not spermine, increased 2-3-fold in liver, but were unchanged in muscle (Table 2). Muscle is a tissue not directly involved in vitellogenesis [14, 28] and will therefore not change its polyamine concentration under the influence of the estrogen.

After estradiol treatment the spermidine^{3+} cation showed a stronger *in vitro* condensating effect on chromatin than Mg^{2+} [29]. The condensating action *in vitro* of the polyamine extended to the activated gene regions, demonstrating that the positively charged cations were involved in the organization of chromatin probably by stabilizing its structure (Fig. 6).

In summary, the cellular and molecular events that occur in the vitellogenin-producing liver provided an ideal system for the study of several fundamental biological processes in the nucleus. The abundantly transcribed vitellogenin gene(s) were used to investigate hormone- and tissue-specific gene expression, and to analyze the structural organization of the chromatin connected with the gene activation. Large chromatin domains became condensed and the expression of genes not required for vitellogenesis diminished. Thus, during the short period of egg yolk formation the synthesis of proteins involved in vitellogenesis is favoured. The condensation of chromatin was paralleled by an increase in polyamines required to stabilize the condensed structures. The disperse chromatin revealed the association of specific proteins of which so far only the 21 kDa protein has been characterized.

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