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Structure of the Lysozyme Gene and Expression in the Oviduct and Macrophages

H. Hauser, T. Graf, H. Beug, I. Greiser-Wilke, W. Lindenmaier, M. Grez, H. Land, K. Giesecke, and G. Schütz

A. Introduction

Lysozyme, a specific differentiation marker for the myeloid lineage of hematopoietic differentiation in mammals (Hansen 1974), is also found in chicken hematopoietic cells (macrophages) (H. Hauser, unpublished work). In the mature hen oviduct lysozyme is produced as one of the major egg white proteins under the control of steroid hormones (Palmiter 1972; Hynes et al. 1979; Schutz et al. 1978).

We show that in chicken macrophages and oviduct cells the same mRNAs are transcribed from a single lysozyme gene. In macrophages, however, the lysozyme gene is expressed at a much lower rate and does not seem to be under the control of steroid hormones.

B. Organization of the Chicken Lysozyme Gene

The lysozyme structural gene is interrupted by three introns on the genomic DNA (Fig. 1). Hybridization data indicate that this gene is probably represented only once per haploid genome (Nguyen-Huu et al. 1979; Lindenmaier et al. 1980). Restriction mapping of sperm, oviduct, and erythrocyte DNA leads to the conclusion that the organization of the coding, intervening, and surrounding sequences of the lysozyme gene are identical or very similar in these tissues. This indicates that gene rearrangement during differentiation does not occur (Sippel et al. 1980).

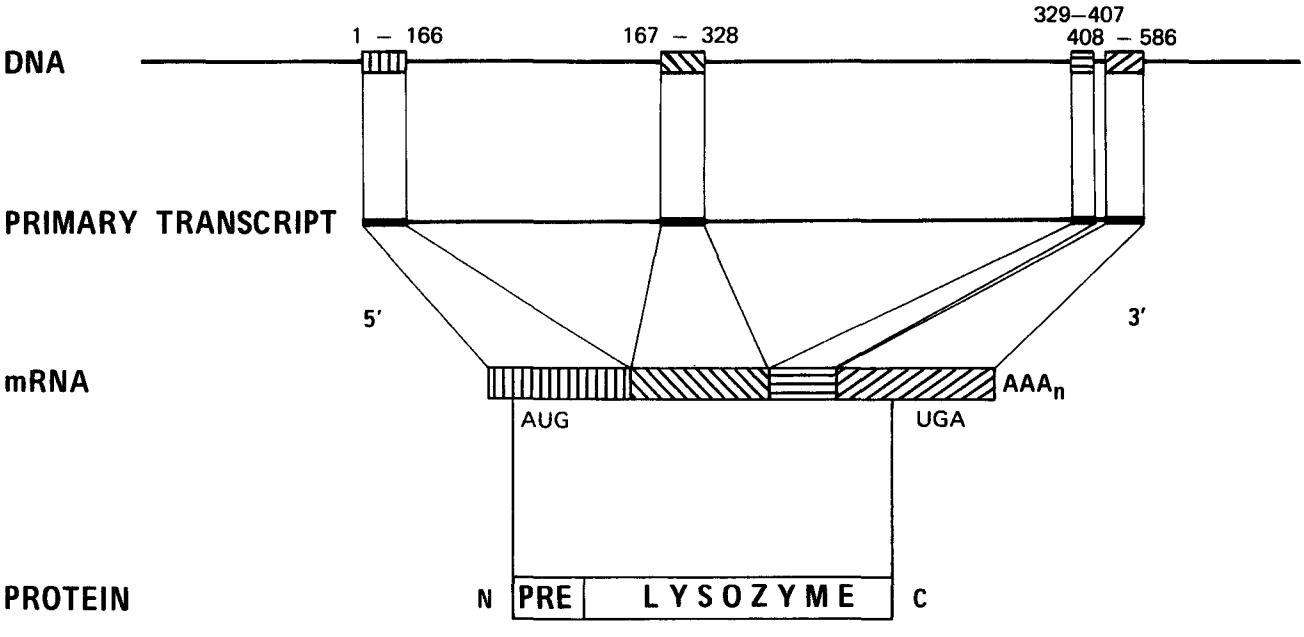

Fig. 1. Schematic representation of the structure and expression of the chicken lysozyme gene

C. Expression of the Chicken Lysozyme Gene in Oviduct

Treatment of young chicks with estrogen leads to the differentiation and proliferation of the oviduct cells and the production of the egg white proteins and their specific mRNAs. Hormone withdrawal leads to deinduction of the egg white protein specific mRNAs. Readministration of estrogen to withdrawn animals reinduces the accumulation of the specific egg white protein mRNAs. This accumulation is due to changes in the rate of transcription of the egg white protein genes and selective stabilization of their mRNAs (Hynes et al. 1979; Schütz et al. 1978; Palmiter and Carey 1974; Swaneck et al. 1979).

To understand the mechanism by which the functional lysozyme mRNA is generated from the split gene, we have analyzed oviduct nuclear RNA containing lysozyme-specific sequences by size analysis (Northern hybridization), S1 nuclease mapping, and electron microscopy. Active mRNA is produced by sequential splicing of the intervening sequences from the primary transcript. This can be seen in the electron micrographs presented in Fig. 2. From these data we could show that the 5′ and 3′ end of lysozyme pre-mRNA appear to be conserved during mRNA maturation.

D. Expression of the Chicken Lysozyme Gene in Macrophages

Lysozyme is also synthesized in chicken macrophages. The synthesis, however, does not seem to be under the control of steroid hormones (H. Hauser, unpublished work). To compare the lysozyme mRNA from oviduct and macrophages we have hybridized lysozyme cDNA obtained from highly purified oviduct lysozyme mRNA to poly(A) containing RNA from macrophages. Size analysis of the hybrids after S1 digestion indicates that macrophage lysozyme mRNA is homologous to

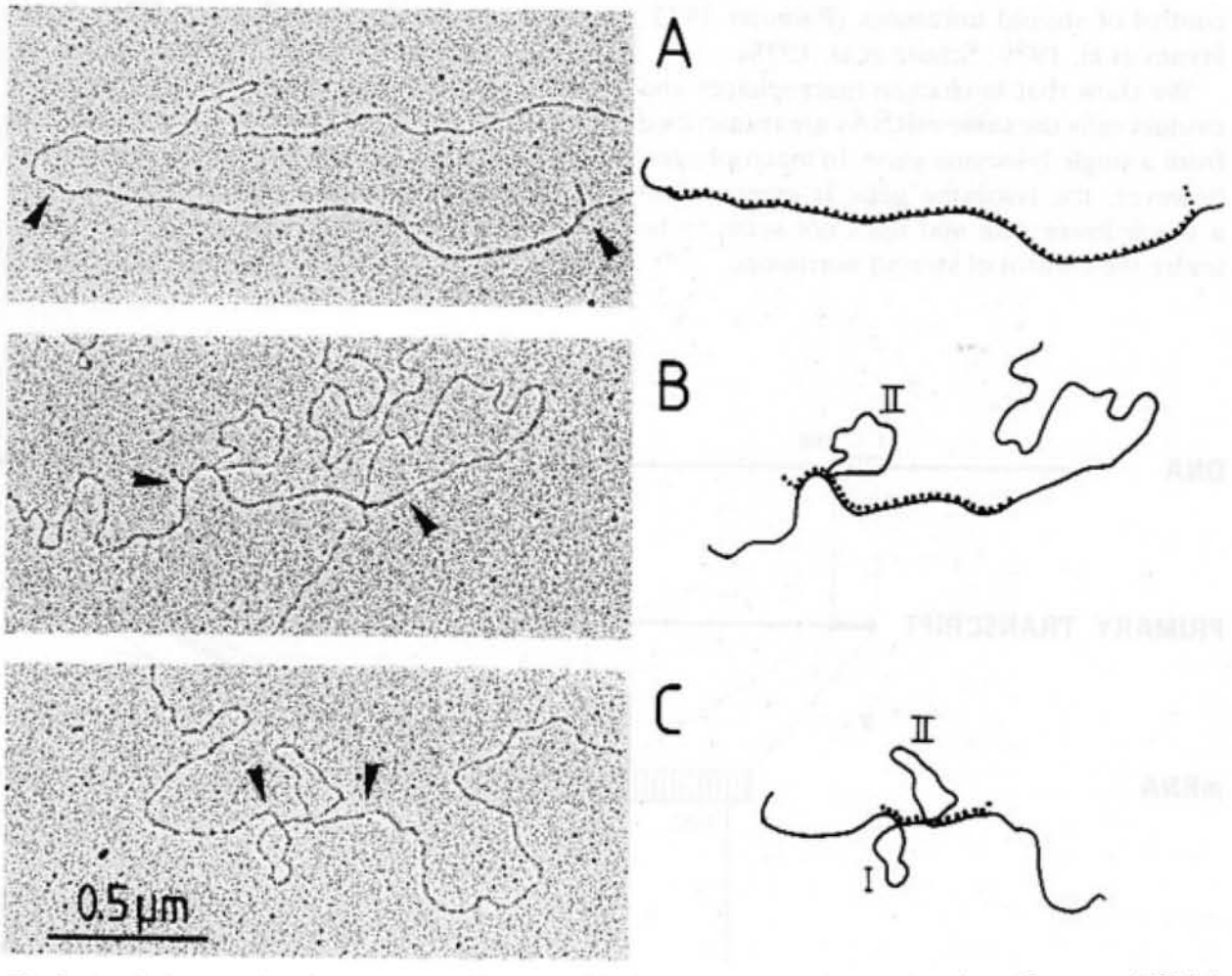

Fig. 2. Analysis of nuclear lysozyme specific-RNA. Nuclear RNA was hybridized to cloned lysozyme DNA in conditions favorable for the formation of hybrids but preventing DNA-DNA duplex formation (Lindenmaier et al. 1980)

the lysozyme cDNA prepared from oviduct mRNA (Fig. 3). Together with the data from chromosomal gene analysis these data indicate that the chicken macrophage lysozyme mRNA is transcribed from the same gene as in the oviduct.

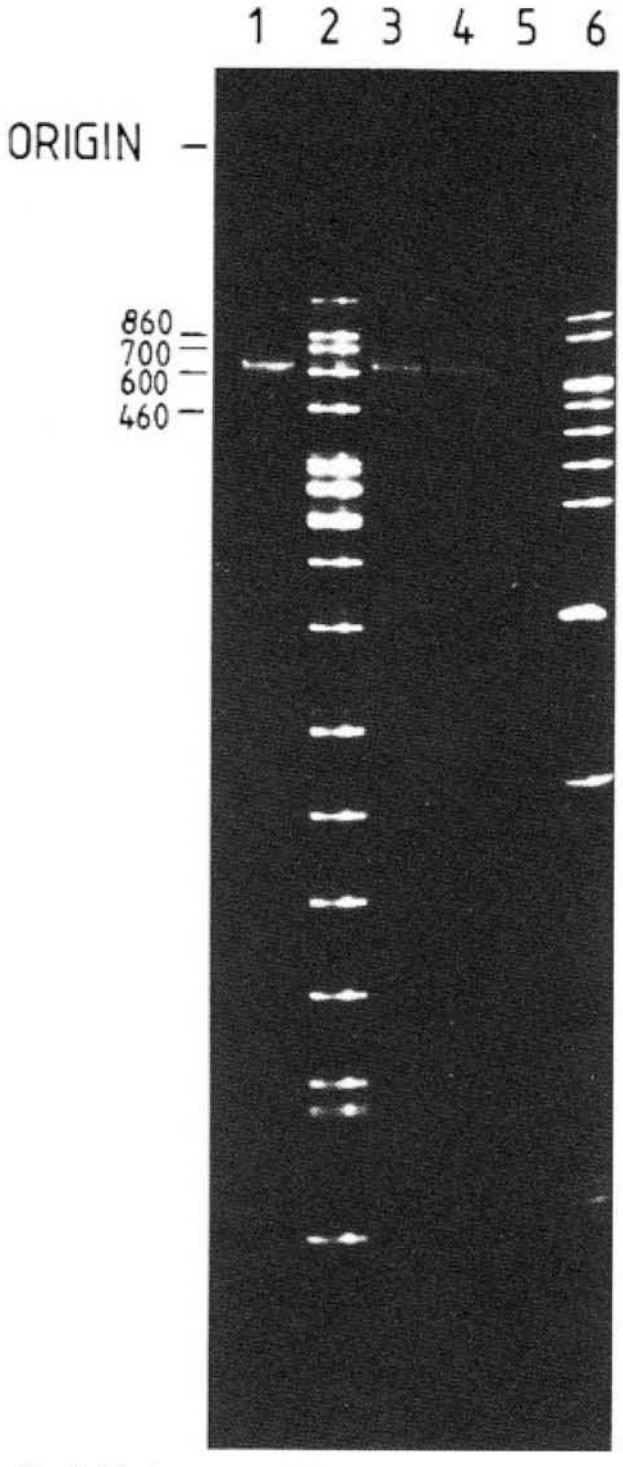

Fig. 3. The lysozyme mRNA sequence isolated from the oviduct is contained in lysozyme mRNA from macrophages. Total poly(A)-containing RNA from macrophages *(Lane 1)* and oviduct *(Lane 3)* were hybridized to full-length cDNA prepared from lysozyme mRNA purified from the oviduct. The hybrids were treated with S1-nuclease and electrophoresed on a DNA sequencing gel. *Lanes 4* and *5*, cDNA alone before and after S1-nuclease digestion; *Lanes 2* and *6*, DNA size markers

The avian retroviruses AMV and MC29 transform preferentially cellular precursors of myeloblasts and macrophages, respectively, and appear to block their differentiation (Graf and Beug 1978). Specific markers of myeloid differentiation indicate that the transformed cell lines are promyeloblasts (AMV-transformed) and promacrophages (MC29-transformed) (Beug et al. 1979). Bone marrow cell lines transformed by AMV and MC29 were found to secrete different amounts of lysozyme independently of the addition of hormones (Table 1).

The AMV transformed cell line can be induced by exposure to Na-butyrate so synthesize 20-fold higher levels of lysozyme than untreated cells (Fig. 4). Quantitations of the lysozyme mRNA is in progress to analyze whether the induction of the enzyme is regulated at the level of transcription.

The results described provide a new model to compare the different molecular mechanisms involved in regulation of the chicken lysozyme gene. In oviduct cells the lysozyme gene is expressed under the control of steroid hormones. In mature macrophages, however, it is expressed constitutively. Induction of expression during cell differentiation can be studied in myeloid cell lines.

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Table 1. The lysozyme protein content in the cell supernatant was determined by its enzymatic activity with the lysozyme plaque assay 24 h after medium change. The mRNA concentrations were determined by cDNA excess hybridization with cDNAs from purified lysozyme and ovalbumin mRNA. Lysozyme gene expression in different cells

Cell type	Source	Lysozyme protein	Lysozyme mRNA (molecules/cell)	Ovalbumin mRNA (molecules/cell)
Tubular gland cells	Laying hen oviduct	+++	29,000	80,000
Tubular gland cells	Hormone-withdrawn chicken oviduct	N.D.	5	5
Macrophages primary culture	Chicken	+	100–300	N.D.
MC29 (RAV-2) HBC1	MC29-transformed chicken bone marrow cells (cell line)	+	30–60	N.D.
AMV DU 1765	AMV-transformed chicken bone marrow cells (cell line)	+	5–20	<0.01
AEV6 C-2	AEV-transformed chicken bone marrow cells (cell line)	–	<0.01	<0.01

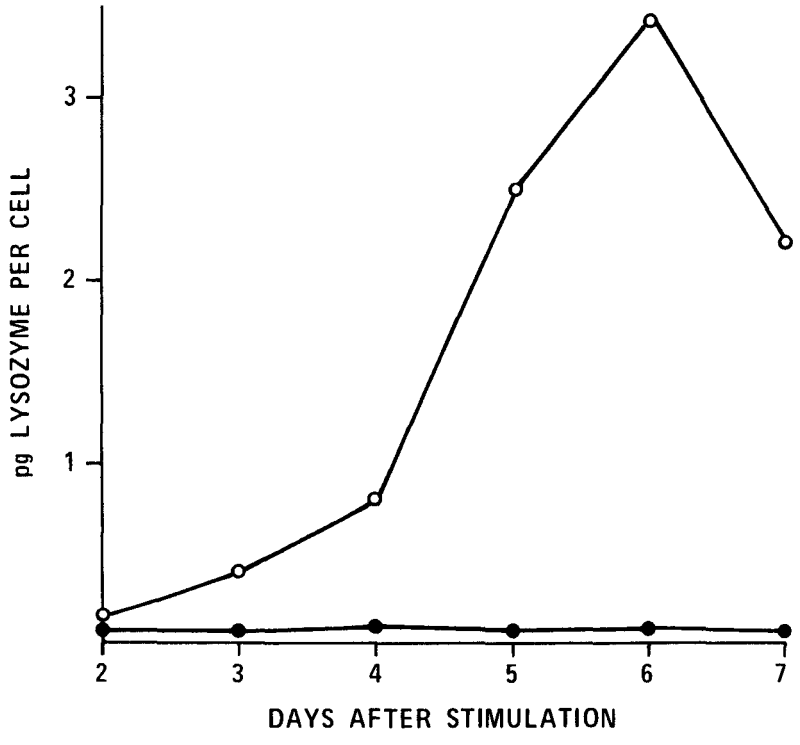

Fig. 4. Stimulation of lysozyme production in AMV-transformed cells by Na-butyrate. AMV DU 1765 cells (see also Table 1) were grown in the presence of 1 m*M* Na-butyrate (o). The cell density was adjusted every two days to 10^6 cells/ml. 50% of the culture medium was renewed every 2 days. The lysozyme protein content in the cell supernatant was determined by its enzymatic activity with the lysozyme plaque assay

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