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# Vertebrate histone gene transcription occurs from both DNA strands

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## ABSTRACT

Three clones containing vertebrate histone genes, two from chicken and one from human have been investigated. DNA from each recombinant within λ Charon 4A has been strand-separated on agarose gels in the presence of poly r(UG). Southern transfer analysis with histone cDNA indicates that in each case transcription occurs from both strands. These results argue against the possibility of polycistronic transcription of these vertebrate histone genes.

## INTRODUCTION

The nucleosome is the basic unit of eukaryotic chromosomes and requires stoichiometric amounts of the four core histones (1). A polycistronic unit encompassing these genes could satisfy stoichiometry at the transcriptional level. (H1 needs separate consideration). Because sea urchin (2) and *Drosophila* (3) histone genes are clustered in tandem arrays the notion of polycistronic transcription has appeal. However this is not supported by experimental evidence. In sea urchin, histone genes are transcribed from one strand, but it is likely that independent initiation occurs (4) and in *Drosophila*, the histone genes are transcribed from both strands (3). This is also true for yeast (5).

Here we present evidence that both chicken and human histone genes are transcribed from both DNA strands. Based on the method of Goldbach *et al.* (6), we have optimised conditions for the separation of complementary strands of different histone gene recombinants cloned in λ Charon 4A. After Southern transfer the strands are probed with (single-stranded) histone

cDNA. Two different chicken histone clones (λCH-01 and λCH-02) and a human histone clone (λHH-01) have been investigated. We have reported data on λCH-01 before (7). More extensive examination of this clone and of λCH-02 (to be reported elsewhere) shows that the organisation of chicken histone genes is complex and does not support our earlier indications of a simple repeating unit (8). The experimental approach used here gives basic information about transcription of clustered genes but does not require detailed knowledge of gene arrangement. Our results do not support an earlier contention for polycistronic transcription for vertebrate (human) histone genes (9) and is in agreement with the UV irradiation studies (10) suggesting that transcription of HeLa histone genes occurs independently.

## MATERIALS AND METHODS

### Recombinant Clones

The insert (0.6 Kb) from a cDNA clone derived from chicken α-globin mRNA (pCGα-1, ref. 11) was re-cloned into a λ Murray vector (λ728). The recombinant is designated λ728(α). Clone λ55 contains an entire histone gene repeat (7 Kb) from the sea urchin Echinus esculentus and was kindly provided by Professor K. Murray. The chicken histone clone λCH-01 has been reported previously and λCH-02 (with a different restriction enzyme pattern and gene arrangement) was selected by the same procedures (7). The inserts are 14.0 Kb and 14.6 Kb respectively. The human histone clone λHH-01 was selected from a human recombinant library in λ Charon 4A (kindly provided by Dr. T. Maniatis). The insert is 14.3 Kb and DNA sequencing has verified it as a histone clone (to be reported elsewhere).

### Complementary Strand Separation

Goldbach et al. (6) have described the basic method for strand-separation of recombinant DNA within 'phage λ. Differential binding of poly r(UG) to the vector DNA (12) enhances separation of the heat-denatured strands in 0.3% agarose gels. Nevertheless optimal conditions for strand separation will vary

from one recombinant to another depending on the size, orientation and composition of the insert DNA. The ratio of poly r(UG) to DNA had a major effect on strand resolution. For example for λ55 with an insert of 7 Kb, excellent resolution was obtained with a ratio of 8:1 (see Fig. 1, track E) whereas at a ratio of 16:1, the resolution was very poor (data not shown). The optimal ratios for the recombinants investigated here were as follows:

| Recombinant | Ratio (w/w) poly r(UG) to DNA |
|---|---|
| λ728(α) | 19:1 |
| λ55 | 8:1 |
| λCH-01 | 20:1 → 25:1 |
| λCH-02 | 10:1 |
| λHH-01 | 8:1 → 16:1 |

## Resolution of Single-stranded Species by Agarose Gel Electrophoresis

After heat denaturation and complexing with an appropriate ratio of poly r(UG) [w/w] the sample was rapidly chilled to 4°C to prevent strand reannealing and was then loaded onto a 0.3% (w/v) horizontal agarose gel. Electrophoresis was carried out for 10 hours at 4°C (under constant current) in 6 mM Tris-HCl, 7.2 mM $NaH_2PO_4$ and 0.2 mM EDTA, pH 7.7 buffer as previously described (13).

## Detection of Strands

Either single or double-stranded DNA was detected in gels by UV illumination after staining with ethidium bromide. Strands complementary to cDNA were detected by standard procedures (14). The cDNA preparations used as 'probes' were either chicken globin cDNA (15) or chick histone cDNA (8). Hybridization was at 42°C in 2 x SSC/50% formamide (16 h) and washing of filters was carried out as described by Wahl *et al.* (16).

## RESULTS AND DISCUSSION

Recombinant DNA technology allows the isolation of unique regions of a genome. If the vector used is λ 'phage there is

the added possibility of separating the complementary strands with sufficient resolution to investigate basic information about the transcription of clustered genes. We carried out initial experiments with a single globin gene recombinant (λ728(α)) and the more complex recombinant clone λ55, which contains a copy of each of the five sea urchin histone genes in a 7 Kb insert. In each case repeatable strand separation was achieved (for details see Fig. 1). When DNA was transferred to

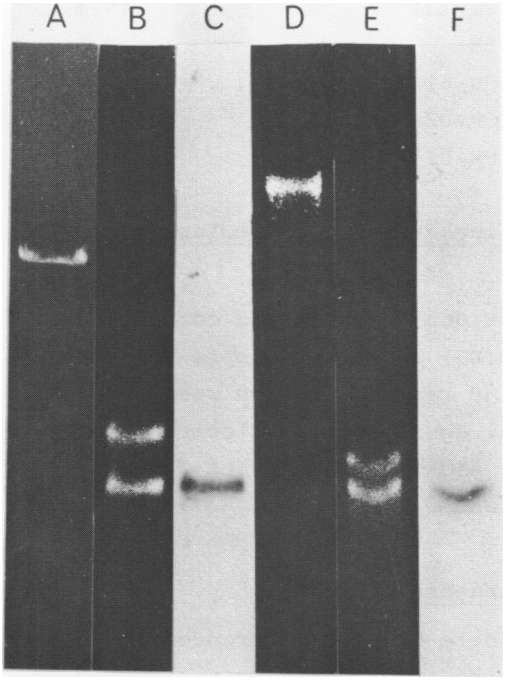


Fig. 1. Characterisation of electrophoresed λ728(α) and λ55 DNA denatured in the presence of poly r(UG). The figure shows a 0.3% agarose gel stained with ethidium bromide, aligned with autoradiographs of Southern strips.

A. ds λ728(α)DNA
B. ss λ728(α)DNA
C. ss λ728(α) after hybridization with $^{32}$P-labelled globin cDNA
D. ds λ55 DNA
E. ss λ55 DNA
F. ss λ55 after hybridization with $^{32}$P-labelled histone cDNA.

Inputs of DNA, 0.5 μg per slot with a ratio of poly r(UG) to DNA of 19:1 and 8:1 in B and E respectively.

nitrocellulose and probed with either chicken globin cDNA or chicken histone cDNA, only one strand in each case hybridized with cDNA. These control experiments demonstrated that the assay was reliable and that negligible strand contamination occurred.

The result with λ55 is the more significant. Although a heterologous probe was used in this case, the conserved nature of histones and of their genes allows cross-hybridization between chicken and sea urchin of the four core histone gene sequences (our unpublished data). It is apparent therefore that histone

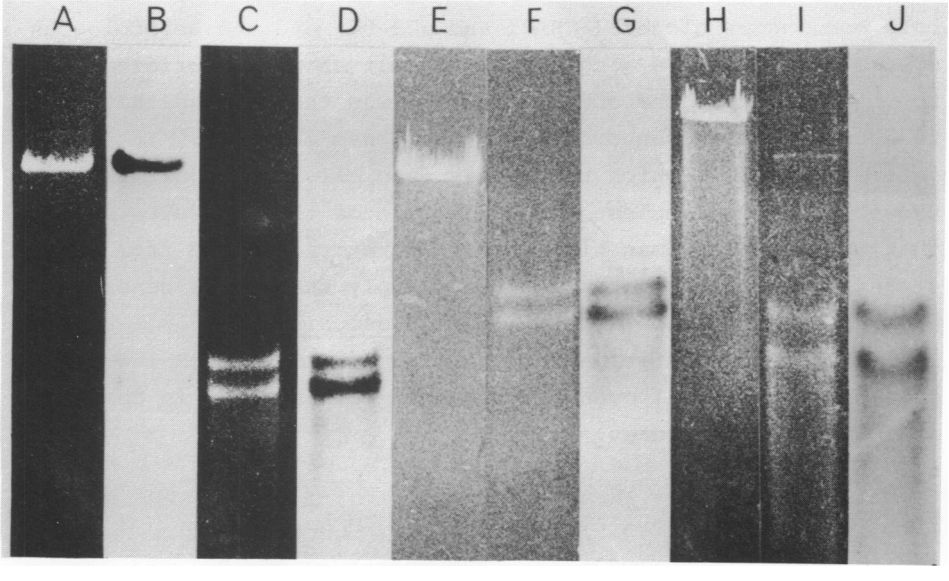


Fig. 2. Characterisation of electrophoresed λCH-01, λCH-02 and λHH-01 DNA denatured in the presence of poly r(UG). DNA is detected with ethidium bromide and Southern strips with $^{32}$P-labelled chick histone cDNA.

A. ds λCH-01
B. ds λCH-01 hybridized with cDNA
C. ss λCH-01
D. ss λCH-01 hybridized with cDNA
E. ds λCH-02
F. ss λCH-02
G. ss λCH-02 hybridized with cDNA
H. ds λHH-01
I. ss λHH-01
J. ss λHH-01 hybridized with cDNA.

Input of DNA, 0.5 μg per slot with a poly r(UG) ratio of 20:1, 10:1, 16:1 in C, F and I respectively.

gene transcription from E. esculentus (λ55) occurs from one strand. This is to be expected since the organisation of these genes closely parallels the arrangement seen in the other sea urchin species P. miliaris and S. purpuratus (K. Gross, personal communication). In these latter two species it is known that histone genes are transcribed from the same strand (17).

A second series of experiments was carried out with three vertebrate histone gene recombinants (see 'Materials and Methods'). In each case, despite the relatively large inserts, strand separation was achieved (Fig. 2). After transfer of DNA to nitrocellulose and hybridization with chick histone cDNA, both homologous clones (λCH-01 and λCH-02) and the heterologous system (λHH-01) gave a consistent result showing hybridization to both strands. The cDNA probe used was the same as that used to hybridize with sea urchin histone genes (Fig. 1). It is derived from mRNA which translates into all five major histone types (8). The simplest interpretation of these results is that chicken and human histone genes are transcribed from both strands. This does not necessarily imply that these genes are organised in the same fashion as in Drosophila.

These results strongly support a model in which independent transcriptional events occur from both strands in chicken and human histone genes.

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