6-1992

Promoter sequence containing (CT)n.(GA)n repeats is critical for the formation of the DNase I hypersensitive sites in the Drosophila hsp26 gene

Qin Lu  
*Washington University in St. Louis*

Lori L. Wallrath  
*Washington University in St. Louis*

Barbara D. Allan  
*Washington University in St. Louis*

R Glaser

J Lis

See next page for additional authors

Follow this and additional works at: [https://openscholarship.wustl.edu/bio_facpubs](https://openscholarship.wustl.edu/bio_facpubs)

Part of the [Biology Commons](https://openscholarship.wustl.edu/bio_facpubs)

**Recommended Citation**

Lu, Qin; Wallrath, Lori L.; Allan, Barbara D.; Glaser, R; Lis, J; and Elgin, Sarah C.R., "Promoter sequence containing (CT)n.(GA)n repeats is critical for the formation of the DNase I hypersensitive sites in the Drosophila hsp26 gene" (1992). *Biology Faculty Publications & Presentations*. 219. [https://openscholarship.wustl.edu/bio_facpubs/219](https://openscholarship.wustl.edu/bio_facpubs/219)

This Article is brought to you for free and open access by the Biology at Washington University Open Scholarship. It has been accepted for inclusion in Biology Faculty Publications & Presentations by an authorized administrator of Washington University Open Scholarship. For more information, please contact digital@wumail.wustl.edu.
Promoter Sequence Containing $(CT)_n \cdot (GA)_n$ Repeats is Critical for the Formation of the DNase I Hypersensitive Sites in the Drosophila hsp26 Gene

Qin Lu, Lori L. Wallrath, Barbara D. Allan, Robert L. Glaser, John T. Lis and Sarah C. R. Elgin

Department of Biology and Division of Biology and Biomedical Sciences Washington University, St Louis, MO 63130, U.S.A.

Section of Biochemistry, Molecular and Cellular Biology Cornell University, Ithaca, NY 14853, U.S.A.

(Received 31 October 1991; accepted 24 February 1992)

We have analyzed P-element-transformed lines carrying hsp26/lacZ transgenes with various deletions and substitutions within the Drosophila melanogaster hsp26 promoter region in order to identify the sequences required for the formation of the DNase I hypersensitive sites (DH sites). DH sites are generally found associated with promoters and enhancer elements of active and inducible eukaryotic genes, and are thought to be nucleosome-free regions of DNA that interact with regulatory proteins and the transcriptional machinery. There are two major DH sites located within the promoter region of the hsp26 gene, centered at -50 and at -350 (relative to the hsp26 transcription start site). The sequences from -135 to -85, which contain $(CT)_n \cdot (GA)_n$ repeats, contribute significantly to the formation of the DH sites in the hsp26 promoter region. Deletion or substitution of this $(CT)_n$ region drastically reduces the accessibility of the DNA at these sites to DNase I. This reduction in accessibility was quantified by measuring the susceptibility of the DNA within nuclei to cleavage at a restriction site within the DH site. In addition to the $(CT)_n$ region and the promoter at -85 to +11 (region P), one of two other regions must be present for effective creation of the DH sites: sequences between -351 and -135 (region A), or sequences between +11 and +632 (region D). Disruption of the wild-type chromatin structure, as assayed by the loss of accessibility to the DH sites, is correlated with a decrease in inducible transcriptional activity, even when the TATA box and heat shock regulatory elements are present in their normal positions.

Keywords: chromatin structure; DH sites; gene expression; CT binding protein/GAGA transcription factor; heat shock genes

1. Introduction

Gene expression in the eukaryotic cell is highly constrained by the packaging of DNA into a complex chromatin structure. Upon replication, DNA and histone are rapidly assembled into a nucleosome array, forming the 100 Å (1 Å = 0.1 nm) chromatin fiber, and are subsequently packaged into higher-order structures. Early studies on the organization of nucleosome arrays demonstrated that sites hypersensitive to digestion by nucleases such as DNase I (DH sites) were generally observed in association with active and inducible genes (Varshavsky et al., 1978; Wu et al., 1979). DH sites are commonly found at the promoters, upstream regulatory sites, and enhancers (whether 5' or 3') of these genes (Wu, 1980; for a review, see Gross & Garrard, 1988). Biochemical analyses of DH site regions (McGhee et al., 1981), as well as footprinting of their chromatin structure in isolated nuclei

† Present address: Department of Embryology, Carnegie Institution of Washington, 115 West University Parkway, Baltimore, MD 21210, U.S.A.

\* Abbreviations used: DH sites, DNase I hypersensitive sites; HSE, heat shock element; HSF, heat shock factor; kb, 10^3 bases or base-pair(s); bp, base-pair(s).
The features of chromatin that dictate the position of DH sites, gaps in the nucleosome array, may play critical roles in permitting certain protein-DNA interactions and in blocking others. How then are DH sites specified? The heat shock genes of *Drosophila melanogaster* are activated within minutes in response to a temperature shift or other stress; they exhibit a chromatin structure poised for transcriptional induction (Rougvie & Lis, 1988). Studies utilizing both ultraviolet photo-crosslinking and run-on transcription assays have shown that RNA polymerase II is associated with the *hsp70* and *hsp26* promoter regions in *Drosophila* tissue culture cells prior to gene activation (Gilmour & Lis, 1986; Rougvie & Lis, 1988, 1990). Identified regulatory elements of *hsp26* located upstream from the transcription start site are the TATA box, proximal and distal heat shock elements (HSE) at positions −60 (HSE 1 and 2) and −350 (HSE 6), and the (CT)₆ (GA)₆ repeats (Fig. 1). Extensive analyses of the upstream regulatory region of *hsp26* has demonstrated that the sequences from −351 to +632 are sufficient to mediate a heat shock response similar to that of the wild-type gene. Both the proximal HSE (HSE 1 and 2) and the distal HSE (HSE 6) are critical for heat shock induction; however, other recognizable elements with weak homology to HSE (HSE 3 to 5) located between −100 and −300, and HSE 7, do not appear to contribute significantly to heat shock induction (Cohen & Meselson, 1985; Pauli et al., 1986; Simon & Lis, 1987; Glaser et al., 1990). Functional regions discussed in this paper are marked by brackets above the map, while chromatin structural features determined earlier are marked below. RI, EcoRI; RV, EcoRV; X, XhoI.

High-resolution genomic footprinting analysis has revealed that within the broad proximal DH site, the TATA and (CT)₆ regions are both protected in non-heat-shocked nuclei isolated from embryos, indicating that proteins are bound specifically to these regulatory elements prior to induction (Thomas & Elgin, 1988). In contrast, prior to heat shock, the two HSEs show a pattern of DNaI cleavage similar to that of naked DNA (Thomas & Elgin, 1988), indicating that these sites are completely accessible. After heat shock, DNaI footprinting indicates that these two HSEs are occupied by the heat shock factor (HSF; Topol et al., 1985; Wu et al., 1987; Thomas & Elgin, 1988). Analysis of the chromatin structure of *hsp26* with methyldimidium-propyl-EDTA-Fe(II) and DNaI has revealed that the promoter region of *hsp26* is organized such that the HSEs lie within proximal and distal DH sites, while much of the remaining region between the DH sites is occupied by a positioned nucleosome (Fig. 1; Cartwright & Elgin, 1986; Thomas & Elgin, 1988). The wrapping of the DNA around this nucleosome may bring the proximal and distal HSEs into close proximity, facilitating interaction between the HSEs and the TATA-binding complex after heat shock induction.

While the roles of the various cis-acting sequence elements discussed above in heat-induced expression of *hsp26* have been extensively studied, the sequence requirements for setting up the DH sites, which maintain the HSEs in an open, accessible conformation, have not been delineated. Interactions of DNA with histones and non-histone chromosomal proteins are clearly involved in the process of DH site formation. In vitro studies of chromatin assembly and transcription have suggested that in some instances, binding of TFII D to the TATA box is sufficient to drive formation of an appropriate transcription complex in competition with nucleosome formation, while in other instances, ancillary proteins are necessary (Workman et al., 1990; Becker et al., 1991). We have investigated the sequence requirements for the formation of DH sites in the *hsp26* promoter by analyzing the chromatin structure generated in vivo for 12 variant *hsp26/lacZ* constructs with permutations within the promoter. These constructs were inserted into the *Drosophila* genome by P-element-mediated transformation (Rubin & Spradling, 1982). Chromatin structure of the transgenes was determined using larval nuclei from at least two independently transformed lines. Our results indi-

**Figure 1.** Map of *hsp26/lacZ* fusion gene. The structure of cP26Z-2kb is shown. The *hsp26* sequence from −1917 to +632 is fused in-frame to the *E. coli lacZ* gene as described (Glaser & Lis, 1990). Restriction sites shown on the top map are those sites giving marker fragments used in the chromatin structure analysis. The 1.1kb DNA fragment used as probe in the indirect end-labeling analysis is the EcoRI–EcoRV fragment isolated from plasmid pMC1753, indicated as a box (Casadaban et al., 1983). The partial restriction map of the *hsp26* sequence (−1917 to +632) is enlarged below with the (CT)₆ regions, the TATA box and the 2 required HSEs (numbers 1, 2 and 6, filled boxes) diagrammed. HSEs 3 to 5 (shown as open boxes) are not involved in heat shock induction. HSE 7 is accessible in isolated nuclei, but is not required for heat shock induction (Pauli et al., 1986; Simon & Lis, 1987; Thomas & Elgin, 1988; Glaser et al., 1990). Functional regions discussed in this paper are marked by brackets above the map, while chromatin structural features determined earlier are marked below. RI, EcoRI; RV, EcoRV; X, XhoI.
cate that the (CT)$_n$ region plays a central role in generating the DH sites; adjacent stretches of DNA are also required for appropriate DH site formation. Generation of the DH sites appears to be essential for subsequent gene activation.

2. Materials and Methods

(a) Plasmid construction and germline transformation

The plasmid constructs and P-element-transformed fly lines containing cP26Z-2kb (cP26Z-36A and cP26Z-84D), cP26ZAX, cP26Z-70A and cP26Z-70B transgenes have been described by Simon & Lis (1987). All other constructs used in this study are described by Glaser et al. (1990) and Glaser & Lis (1990). Two independent lines showing average β-galactosidase activity for a particular construct (as quantified by CPRG assays) were used for chromatin structure determination. CPRG assays were carried out as described (Simon & Lis, 1987). The structures of the various hsp26/lacZ transgenes were confirmed by genomic restriction mapping of the DNA from the transformed lines (data not shown).

(b) Preparation of larval nuclei, DNase I partial digestion and indirect end-labeling analyses

Transformed fly lines were raised at 23°C. Mid-third instar larvae were collected from the walls of the bottles. Non-heat-shocked larvae were collected, frozen in liquid nitrogen and then stored at -70°C. For heat shock induction, the collected larvae were transferred to a 15 ml glass beaker kept humid with a disk of wet filter paper. The beaker was covered with aluminum foil and placed in an incubator at 37°C for 45 min. The heat-shocked larvae were frozen in liquid nitrogen and stored at -70°C.

Larvae (1 g) were pulverized in liquid nitrogen using a mortar and pestle. Then 3 ml of buffer Β solution (60 mM-KCl, 15 mM-NaCl, 1 mM-EDTA, 0.1 mM-EGTA, 15 mM-Tris·HCl (pH 7.4), 0.15 mM-spermine, 0.5 mM-spermidine, 0.5 mM-dithiothreitol, 0.5% (v/v) Nonidet P-40, 12 mM-EDTA) was added, the homogenate was transferred to a 7 ml ground glass homogenizer and disrupted with 5 strokes of the pestle. The homogenate was then transferred to a 15 ml Dounce homogenizer and disrupted with 10 strokes with a pestle (type B). The homogenate was filtered through 2 layers of 89 mm nylon mesh cloth. Then 2 ml of buffer Β was used to rinse the homogenate through the same 2 layers of nylon mesh cloth. The mixture was loaded over 1 ml of buffer AS (60 mM-KCl, 15 mM-NaCl, 1 mM-EDTA, 0.1 mM-EGTA, 15 mM-Tris·HCl (pH 7.4), 0.15 mM-spermine, 0.5 mM-spermidine, 0.5 mM-dithiothreitol, 0.3 mM-sucrose) and centrifuged in a Sorval HB4 swinging bucket rotor at 4°C, 3000 revs/min for 5 min. The recovered pellet was dispersed in 3 ml of buffer AS by 5 strokes with a pestle (type A) in a 7 ml Dounce homogenizer, and the mixture was loaded over 1 ml of buffer AS, and centrifuged as before. The recovered pellet was dispersed in 3 ml of buffer Α (60 mM-KCl, 15 mM-NaCl, 1 mM-EDTA, 0.1 mM-EGTA, 15 mM-Tris·HCl (pH 7.4), 0.15 mM-spermine, 0.5 mM-spermidine, 0.5 mM-dithiothreitol, 0.3 mM-sucrose) and centrifuged in a Sorval HB4 swinging bucket rotor at 4°C, 3000 revs/min for 5 min. The recovered pellet was dispersed in 3 ml of buffer Α by 5 strokes with a pestle (type A) in a 7 ml Dounce homogenizer, and the mixture was loaded over 1 ml of buffer AS, and centrifuged as before. The recovered pellet was dispersed in 3 ml of buffer Α (60 mM-KCl, 15 mM-NaCl, 15 mM-Tris·HCl (pH 7.4), 0.25 mM-sucrose, 3 mM-MgCl$_2$, 1 mM-EDTA, 0.05 mM-orotate, 0.05 mM-dithiothreitol) in 7 ml Dounce homogenizer with a pestle (type B). After pelleting at 2000 revs/min for 5 min the nuclei were resuspended in 1 ml of 10 mM-EGTA, 15 mM-Tris·HCl (pH 7.4), 0.15 mM-spermine, 0.5 mM-spermidine, 0.5 mM-dithiothreitol) by 5 strokes in a 7 ml Dounce homogenizer with a pestle (type B). After pelleting at 2000 revs/min for 5 min the nuclei were resuspended in 1 ml of DNase I digestion buffer (60 mM-KCl, 15 mM-NaCl, 15 mM-Tris·HCl (pH 7.4), 0.25 mM-sucrose, 3 mM-MgCl$_2$, 1 mM-EDTA, 0.05 mM-orotate, 0.05 mM-dithiothreitol). Portions of this suspension (200 μl each) were incubated with 0, 1, 2 or 5 μl of DNase I (20,000 units/ml; Worthington Biochemical Corporation, NJ, U.S.A.) on ice for 3 min with agitation. The digestion was stopped by the addition of EDTA to a final concentration of 8 mM. The DNA was purified as described by Wu et al. (1979).

Nuclei were isolated from non-heat-shocked larvae as described for DNase I analysis. The final pellet was resuspended in 1.47 ml of 10 mM-Tris·HCl (pH 7.5), 10 mM-MgCl$_2$, 50 mM-NaCl, 1 mM-dithiothreitol. Phenylmethylsulfonyl fluoride, aprotinin and leupeptin were added to the final concentrations of 0.5 mM, 5 mg/ml and 0.1 unit/ml, respectively. Then 200 units of XbaI (New England Biolabs) were added to 250 μl portions of suspended nuclei and the mixture incubated at 37°C for 45 min. Since the extent of cleavage at the proximal XbaI site of the hsp26 promoter was used to quantify the accessibility of the sequences within the DH site, it was important to insure that the conditions allowed for complete restriction by the enzyme at all available sites. Using increasing amounts of XbaI, we previously determined that incubation with 200 units of XbaI under these conditions gave maximum restriction enzyme digestion (data not shown). The restriction enzyme digestion was terminated by the addition of EDTA to a final concentration of 8 mM and the DNA was purified according to the procedure described by Wu et al. (1979). One-half of each purified sample was cut to completion with 60 units of EcoRV and fractionated on a 1% (w/v) agarose gel, transferred to Nytran membrane and probed with the 1.1 kb fragment of the E. coli lacZ gene (Fig. 1) labeled with [γ-32P]dCTP using random hexamer priming (Feinberg & Vogelstein, 1983). The conditions for hybridization and washing were as described (Cartwright & Elgin, 1986). Membranes were exposed to XAR-5 X-ray film (Kodak) for 12 to 24 h at -70°C using 2 intensifying screens.

(c) Restriction enzyme treatment of isolated nuclei and indirect end-labeling analyses

Nuclei were isolated from non-heat-shocked larvae as described for DNase I analysis. The final pellet was resuspended in 1.47 ml of 10 mM-Tris·HCl (pH 7.5), 10 mM-MgCl$_2$, 50 mM-NaCl, 1 mM-dithiothreitol. Phenylmethylsulfonyl fluoride, aprotinin and leupeptin were added to the final concentrations of 0.5 mM, 5 mg/ml and 0.1 unit/ml, respectively. Then 200 units of XbaI (New England Biolabs) were added to 250 μl portions of suspended nuclei and the mixture incubated at 37°C for 45 min. Since the extent of cleavage at the proximal XbaI site of the hsp26 promoter was used to quantify the accessibility of the sequences within the DH site, it was important to insure that the conditions allowed for complete restriction by the enzyme at all available sites. Using increasing amounts of XbaI, we previously determined that incubation with 200 units of XbaI under these conditions gave maximum restriction enzyme digestion (data not shown). The restriction enzyme digestion was terminated by the addition of EDTA to a final concentration of 8 mM and the DNA was purified according to the procedure described by Wu et al. (1979). One-half of each purified sample was cut to completion with 60 units of EcoRV and fractionated on a 1% (w/v) agarose gel, transferred to Nytran membrane and probed with the 1.1 kb fragment of the E. coli lacZ gene (Fig. 1) labeled as described above. Conditions for the Southern analysis were as published (Wallrath et al., 1990), except 0.8 M-NaCl was used in the prehybridization and hybridization solution rather than 0.1 M-NaCl. Southern blots were exposed to XAR-5 X-ray film (Kodak) for 36 h at -70°C using 2 intensifying screens. The intensities of the bands on the Southern autoradiographs were measured using a scanning densitometer (Molecular Dynamics).

(d) Northern blot analysis

Total RNA was extracted from larvae by the hot phenol method (Jowett, 1986). DNA was removed by digestion with RNase-free DNase I (Boehringer Mannheim Biochemicals). RNA samples were fractionated on a 1% (w/v) agarose gel with formaldehyde using standard methods (Maniatis et al., 1982). RNA was transferred onto Nytran membrane in 20 x SSC (SSC is 0.15 x-
NaCl, 0.15 M-sodium citrate (pH 7.0) and the membrane baked at 80°C in a vacuum oven for 30 min. Prehybridization was carried out in 50% formamide, 5× Denhardt's solution (Denhardt's is 0.02% (w/v) Ficoll, 0.02% (w/v) polyvinylpyrrolidone, 0.02% (w/v) bovine serum albumin), 0.1% (w/v) SDS, 5× SSPE (SSPE is 0.18 M-NaCl, 10 mM-NaPO₄ (pH 7.7), 0.18 M-H₃PO₄, (pH 7.7), 150 μg salmon sperm DNA/ml, at 42°C for 1 h. Hybridization was carried out at 42°C for more than 8 h in 50% formamide, 2.5× Denhardt's, 0.1% (w/v) SDS, 5× SSPE, 150 μg salmon sperm DNA/ml and 10⁶ cts of probe DNA/ml. The probe used was the 1.1 kb lacZ fragment (Fig. 1) labeled as described above. The membrane was washed twice in 6× SSPE, 0.1% SDS for 15 min at room temperature, and twice in SSPE, 0.1% SDS for 15 min at 37°C. The filter was exposed to preflashed XAR5 X-ray film (Kodak) at -70°C with 2 intensifying screens. Subsequently, the membrane was stripped by incubating in 300 ml of stripping solution (5 ml/l-Tris.HCl (pH 8.0), 0.1% (w/v) SDS, 5× SSPE, 15Opg salmon sperm DNA/ml at 42°C for 1 h. Subsequent hybridization was carried out at 42°C for more than 8 h in 5% SDS, 5× SSPE, 0.18 M-H₃PO₄, (pH 7.7), 150 μg salmon sperm DNA/ml, at 42°C for 1 h.

3. Results

(a) The TATA box and downstream sequences, in the absence of sequences from -52 to -372, are insufficient for normal DH site formation

We have analyzed the DH sites of a series of constructs reintroduced into D. melanogaster by P-element-mediated germline transformation (Rubin & Spradling, 1982). To evaluate the role of the TATA box and downstream sequences in forming the DH sites in the hsp26 promoter region, we analyzed a series of transformants containing hsp26lacZ constructs with an upstream internal deletion or reinsertion of regulatory elements (Simon & Lis, 1987). To demonstrate the DH site pattern of the chimeric gene, nuclei isolated from non-heat-shocked larvae of the various transformed lines were treated briefly with DNase I, the DNA was purified and cut to completion with EcoRV, fractionated on an agarose gel, Southern blotted, and the membrane probed with a lacZ fragment (Fig. 1). As shown in Figure 2(c), the control construct, with 1917 bp of upstream sequences (cP267-2kb), shows the characteristic wild-type pattern of two DH sites, one centered over the proximal HSEs (HSE 1 and 2) and the other centered over the distal HSEs (HSE 6 and 7). In cP26ZAX, sequences from -372 to -52 are deleted, which removes the HSEs and (CT)₆₉(GA)₆ sequences, but leaves the TATA box, downstream sequences and sequences upstream from -372 (to -1917) of hsp26 intact (Fig. 2(a)). In transformants carrying the cP26ZAX transgene, the distal DH site was not detectable and the proximal DH site was severely reduced in intensity (Fig. 2(c)). Therefore, in the absence of the HSEs and (CT)₆₉(GA)₆ sequences, the TATA box and downstream sequences are clearly insufficient for the formation of normal DH sites.

While heat shock inducibility of the cP26ZAX transgene is abolished, insertion of a 52 bp sequence containing the hsp70 regulatory region (−38 to −89) in either orientation at the site of the deletion of cP26ZAX (cP26ZAX-70a and cP26ZACT-70b) re-established the heat shock inducibility to 25 to 40% that of wild-type control (Simon & Lis, 1987). Examination of the DNase I hypersensitivity of these transgenes indicates that the insertion of the 52 bp fragment led to formation of a strong DH site (Fig. 2(c)). This short 52 bp sequence has only two known types of elements, HSEs and a short (CT)₆₉ sequence (7 bp). In vitro studies (Zimarino & Wu, 1987) and in vivo studies (Wu, 1984; Cartwright & Elgin, 1986; Thomas & Elgin, 1988) indicate that the HSEs in Drosophila are unoccupied prior to heat shock induction, and therefore unlikely to contribute to DH site formation. The results suggest that the (CT)₆₉ sequence, the only element within the 52 bp fragment of hsp70 known to bind a chromosomal protein prior to heat shock induction, might play a role in DH site formation in this chimera.

(b) Analysis of 5’ deletion mutations suggests that the (CT)₆₉ region and other upstream sequences play a role in DH site formation

The formation of the DH sites in a series of hsp26lacZ fusion gene constructs containing 5’ deletions to −351, −135 and −85 bp was examined. These constructs retain the TATA sequence, the transcription start site and the downstream sequence of hsp26 to +632 (Fig. 3(a)). The cP-351 transgene shows the same proximal and distal DH sites in nuclei from non-heat-shocked animals as does the wild-type hsp26 gene (Fig. 3(c)); see wild-type analysis: Cartwright & Elgin, 1986; Thomas & Elgin, 1988). Previous studies have shown that while two HSEs (HSE 6 and 7) are found in the distal region of the wild-type gene, HSE 7 (centered at −364) is not required for full activity (Cohen & Meselson, 1985; Pauli et al., 1986; Thomas & Elgin, 1988). The cP-135 transgene, which lacks the distal HSEs but contains the (CT)₆₉(GA)₆ sequences, shows a strong proximal DH site. The distal DH site has essentially disappeared, while a very weak site at position about −200 is observed (Fig. 3(c)). Upon deletion to −85 (cP-85), which eliminates the (CT)₆₉ region, the proximal DH site essentially disappears (Fig. 3(c)). These results suggest that the (CT)₆₉ and upstream sequences play an important role in DH site formation.

To quantify the accessibility of the sequences within the proximal DH site, we performed restriction enzyme digestion of isolated nuclei (Fascher et al., 1990; Archer et al., 1991; Reik et al., 1991; Jack et al., 1991). Nuclei from third instar larvae containing either the cP-351, cP-135 or cP-85 transgenes were incubated with XbaI, which cleaves within the proximal DH site. After purification, the chromosomal DNA was restricted to completion with EcoRV, fractionated on an agarose gel, and probed with the 1.1 kb fragment spanning a portion
Figure 2. Chromatin structure in the presence and absence of upstream regulatory elements. Nuclei from non-heat-shocked larvae were treated briefly with DNase I and the purified DNA was subjected to indirect end-labeling analysis as described in Materials and Methods. (a) The constructs analyzed here; the transcription start site at +1 is where the line bends up; the open box indicates the lacZ sequence; the bottom map shows the boundaries of the functional regions discussed. (b) The 52 bp hsp70 cassette used to make the cP26Z-70a and cP26Z-70b constructs. This sequence contains hsp70 HSE 1 and HSE 2, and a short stretch of alternating C and T residues as indicated. The arrow indicates the natural orientation of this sequence in hsp70. (c) DH site formation in non-heat-shocked nuclei. Transgenes are identified above the appropriate lanes. The numbers over each lane indicate the μl of DNase I (20,000 units/ml) added to each 250 μl of nuclear suspension. DH sites are indicated by arrows labeled P DH (proximal DH site) and D DH (distal DH site). The parental band, which is created by EcoRV digestion of DNA not cut by DNase I in the initial digestion, is indicated by an arrow labeled Prt. A doublet band, which has been shown by restriction analysis to be unrelated to this group of constructs, is indicated by an arrow labeled Doublet. In addition to EcoRV digestion, these samples have been digested with AvaI. AvaI does not cut within the parental EcoRV fragment of these constructs, but does cut the unrelated DNA into the observed doublet, which does not then interfere with the DH site analysis. M, markers; RI, EcoRI; RV, EcoRV; X, XbaI.
Figure 3. DH site formation in the 5′ deletion series. Nuclei from non-heat-shocked larvae were treated briefly with DNase I and the purified DNA was subjected to indirect end-labeling analysis as described in Materials and Methods. (a) The constructs used in this Figure, drawn as described for Fig. 2. (b) The sequence within the (CT)_n region. The endpoints of the 5′ deletions are indicated above the sequence. (c) DH site formation in nuclei from non-heat-shocked larvae. DH sites are indicated by arrows labeled P DH (proximal DH site) and D DH (distal DH site). (d) Cleavage susceptibility of the proximal DH site to restriction enzyme XbaI within isolated nuclei. Nuclei were isolated from non-heat-shocked third instar larvae of transformed lines. The restricted DNA was purified and subjected to indirect end-labeling analysis as described in Materials and Methods. Transgenes are identified above the appropriate lanes; other labels are as described for Fig. 2.

of the Escherichia coli lacZ gene (Fig. 1). Cleavage at the XbaI site within the proximal DH site would produce a 1.7 kb band detected by this probe. Cleavage at a second XbaI site, located within the distal DH site, gives a 2.1 kb band. No restriction at the XbaI sites within the hsp26 promoter sequences present in these transgenes gives a EcoRV parental band. The accessibility of the XbaI site within the proximal DH site was quantified as the fraction of the intensity of the 1.7 kb band compared to the total intensity of all the bands within a given lane on an autoradiograph. In transformants carrying the cP-351 transgene, there was 52% restriction at the XbaI site that lies within the proximal DH site (Fig. 3(d)). This is essentially the same as observed for the wild-type hsp26 gene (data not shown). Transformants carrying the cP-135 transgene exhibited a moderate reduction to 38% cleavage at this site, while transformants carrying the cP-85 transgene exhibited a dramatic reduction in accessibility at the proximal XbaI site, showing only 4% cleavage (Fig. 3(d)). Since the majority of the sequences contained between −135 and −85 are (CT)_n, the reduced accessibility of the XbaI site within the proximal DII site of cP-85 was most likely due to the loss of the (CT)_n:(GA)_n repeats.
Figure 4. DH site formation in transgenes lacking the (CT)$_n$ region. Larval nuclei were treated briefly with DNase I and the purified DNA was subjected to indirect end-labeling analysis as described in Materials and Methods. (a) The constructs used in this Figure, drawn as described for Fig. 2. (b) DH site formation in non-heat-shocked (N) and heat-shocked (H) nuclei. In order to visualize the weak bands for the DH sites of transgenes containing the (CT)$_n$ deletion and substitution, the autoradiograph of this panel was overexposed. (c) Susceptibility of the proximal DH site to cleavage by the restriction enzyme XbaI within isolated nuclei from non-heat-shocked larvae. The DNA was purified and subjected to indirect end-labeling analysis. Transgenes are identified above the appropriate lanes. Other labels are as described for Fig. 2.

(c) (CT)$_n$·(GA)$_n$ sequences have a critical role in generating DH sites

Since the above analyses suggested that the (CT)$_n$·(GA)$_n$ sequences might play an important role in the formation of the DH sites, we undertook a chromatin structure analysis of a set of constructs that included an internal deletion of the (CT)$_n$ region (cPACT·GA) as well as sequence substitution at this site (Fig. 4(a)), in nuclei from either non-heat-shocked or heat-shocked larvae. Using DNase I, analysis of the cPACT·GA transgene showed that both the proximal and the distal DH sites were dramatically reduced compared with the cP-351 transgene under both conditions (non-heat-shocked or heat-shocked: Fig. 4(b)). (Note that the distal site appears to be slightly shifted relative to that of the wild-type control due to the removal of the (CT)$_n$ region.) When the (CT)$_n$·(GA)$_n$ sequences were replaced with the same length of a random sequence
Figure 5. DH site formation in transgenes containing multiple deletions. Larval nuclei were treated briefly with DNase I and the purified DNA was subjected to indirect end-labeling analysis as described in Materials and Methods. (a) The constructs used in this Figure, drawn as described for Fig. 2, with the wild-type gene given below for reference. In these constructs the hsp26 sequence from +1 to -135 is replaced by the hsp70 sequence from +89 to +260 as described (Glaser & Lis, 1990). The restriction sites used in the map give marker fragments as indicated. (b) The DH site formation of the hsp26/70/ZacZ constructs made with and without the (CT)_n region. (c) The DH site formation of the hsp26/70/ZacZ transgenes made with and without region A (from -351 to -135) of hsp26. (d) Cleavage susceptibility of the proximal DH site to restriction enzyme XbaI within isolated nuclei. The DNA was purified and subjected to indirect end-labeling analysis. Transgenes are identified above the appropriate lanes. Other labels are as described for Fig. 2.

from salmon sperm DNA (construct cPri), the DH sites appeared similar to those observed for the deletion construct cPACT-GA (Fig. 4(b)). Using the same strategy as described in section (b), above, restriction enzyme analyses of isolated nuclei from non-heat-shocked larvae containing either the cP-351, cPACT-GA or cPri transgene were performed. Transformants carrying either the cPACT-GA or the cPri transgenes showed dramatic reductions in the accessibility for XbaI within the proximal DH site, exhibiting only 8% and 6% cleavage as compared to the 52% cleavage seen for cP-351 (Fig. 4(c)). Thus, removal or replacement of the (CT)_n region has a dramatic effect on DH site formation.

(d) Additional regions that are involved in the formation of the DH sites

The hsp26 sequences from -351 to +632 are sufficient to mediate heat shock response similar to that of the wild-type gene (Cohen & Meselson, 1985; Pauli et al., 1986; Simon & Lis, 1987; Glaser et al., 1990), and to dictate formation of the wild-type chromatin structure (Fig. 2(c)). When hsp26 sequences from -351 to +11 are fused to a different gene, such as Adh, this fusion gene is still heat shock inducible (Cohen & Meselson, 1985). Similarly, fusion of a fragment of hsp26 from -351 to +11 with an hsp70/lacZ construct starting at +89 of hsp70 (cP-351-70Z, Fig. 5(a)) produces a
gene that is heat shock inducible (Fig. 6). DH site analysis of transformants carrying cP-351-70Z revealed a wild-type pattern of DH site formation (Fig. 5(b)).

The proximal DH site is prominent upon analysis of transformants carrying the cP-135 transgene (Fig. 2(c)), which includes the (CT)n,(GA)n repeats and hsp26 sequences downstream to +632; however, fusion of this construct at +11 of hsp26 with hsp70/lacZ at +89 of hsp70 (cP-135-70Z: Fig. 5(a)) results in a dramatic reduction in the formation of the proximal DH site (Fig. 5(c)). These results suggest the presence of an hsp26 downstream element between sequences +11 to +632 (region D). When sequences from −351 to −135 (region A) are not present, region D is apparently required to co-operate with the (CT)n,(GA)n repeats and the TATA sequence to create the DH sites.

The accessibility of XbaI to the XbaI site within the proximal DH site was evaluated for transformants carrying the transgenes with region D changed as described above. Transformants carrying the cP-351-70Z transgene exhibited 46% cleavage (Fig. 5(d)), a value comparable with the similar construct possessing region D (cP-351). Transformants possessing the cP-135-70Z transgene, lacking both regions A and D, exhibited only 9% cleavage at this site (Fig. 5(d)).

The results obtained from the analysis of transformed lines carrying cPACT-70Z, which lacks both the hsp26 (CT)n and the D regions, provide further support for the role of the (CT)n,(GA)n repeats in the formation of DH sites. This transgene retains the A region, HSEs and the TATA box and differs from the parental construct, cP-351-70Z, only in the deletion of the (CT)n region. DNase I has reduced access to the DH sites in lines carrying the cPACT-70Z transgenes relative to lines containing the control transgenes, cP-351-70Z (Fig. 5(b)). This result is consistent with those shown in Figure 4 where the deletion or substitution of the (CT)n region reduced cleavage at the DH sites even in transgenes that have both the A and D regions. Furthermore, restriction enzyme treatment of nuclei isolated from cPACT-70Z transformants results in only 7% cleavage by XbaI within the proximal DH site (Fig. 5(d)).

(e) Formation of DH sites and heat-induced gene expression

Mutations that disrupt the formation of DH sites at hsp26 also impair its heat-induced transcriptional activation. The quantitative measurements of restriction enzyme cleavage at the XbaI site within the proximal DH site described in the preceding sections indicate that the deletion of the (CT)n region causes a decrease of the susceptibility of this site to 12 to 15% that of the wild-type control (compare cPACT-GA and cP-351: Fig. 4 and Table 1). Our previous and present measurements of the heat shock inducibility of hsp26 transgenes show that the (CT)n region is likewise critical for the heat-induced expression of hsp26 (Glaser et al., 1990; Table 1). However, establishment of the DH sites is not sufficient to render hsp26 heat shock inducible. When sequences that are required for transcriptional activation, such as the distal HSE, are absent, the gene is not heat shock inducible even if the DH site(s) are clearly established (see cP-135 in Figs 2 and 6). Nevertheless, if DH sites are absent, as in the (CT), deletions, then the heat shock inducibility is severely limited, even in the presence of essential transcriptional elements such as the TATA box, transcriptional start, and HSEs (see cPACT-GA and cPACT-70Z in Figs 4 to 6 and Table 1).
Summary of restriction enzyme accessibility at the proximal XbaI site and relative expression of transgenes

<table>
<thead>
<tr>
<th>Construct</th>
<th>A (CT)_h</th>
<th>P</th>
<th>D</th>
<th>Cleavage by XbaI (%)</th>
<th>Expression (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>cP-351</td>
<td>+ + + +</td>
<td>52</td>
<td>100</td>
<td></td>
<td></td>
</tr>
<tr>
<td>cP-133</td>
<td>+ + + +</td>
<td>38</td>
<td>17</td>
<td></td>
<td></td>
</tr>
<tr>
<td>cP-65</td>
<td>+ + + +</td>
<td>4</td>
<td>4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>cPACT-GA</td>
<td>+ + + +</td>
<td>8</td>
<td>23</td>
<td></td>
<td></td>
</tr>
<tr>
<td>cPri</td>
<td>+ + + +</td>
<td>6</td>
<td>27</td>
<td></td>
<td></td>
</tr>
<tr>
<td>cP-351-Z0</td>
<td>+ + + +</td>
<td>46</td>
<td>100</td>
<td></td>
<td></td>
</tr>
<tr>
<td>cP-133-Z0</td>
<td>+ + + +</td>
<td>9</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>cPACT-Z0</td>
<td>+ + + +</td>
<td>7</td>
<td>1</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>A (CT)_h</th>
<th>P</th>
<th>D</th>
</tr>
</thead>
<tbody>
<tr>
<td>XbaI</td>
<td>-52</td>
<td>+1</td>
</tr>
<tr>
<td>-531</td>
<td>+1</td>
<td></td>
</tr>
</tbody>
</table>

Table 1

(a) The (CT)_h region is essential for DH site formation.

To assess the role of the (CT)_h region plays in transcriptional control of the hsp26 gene, a number of constructs have been made and analyzed (Glaser et al., 1990; Glaser & Lis, 1990). The results from these analyses have demonstrated that the (CT)_h (GA)_h is a positive regulatory element for both heat shock and developmental expression of this gene. A 66,000 M protein has been identified that binds to and protects the alternating C and T residues in the (CT)_h region in vivo (Gilmour et al., 1989). This CT binding protein appears to be identical with the GABA factor, a transcription factor associated with the Ubx and other promoters (Biggin & Tjian, 1988; Gilmour et al., 1989; H. Granok & S.C.R.E., unpublished results). Footprinting experiments with isolated nuclei show that this region is protected in vitro prior to heat shock (Thomas & Elgin, 1988). Thus, protein-DNA interaction at this site may be a critical step in DH site formation. Alternatively, the importance of the (CT)_h sequence may be due to the structure of the (CT)_h sequence itself. In vitro, these (CT)_h sequences, when present on a supercoiled plasmid, form a triple helix at low pH (Siegfried et al., 1986; J. M. Teare & S.C.R.E., unpublished results). However, this triplex structure could not be detected by DEPC treatment of isolated nuclei (Glaser et al., 1990); whether or not such triplexes form in vitro is a matter of current debate and investigation.

It should be noted that while deletion or substitution of the (CT)_h sequence severely reduces accessibility of the HSE (XbaI site), it does not eliminate the DH site entirely (cPACT-GA and cPri: Fig. 4). The residual DNase I hypersensitivity might be due to a different structure, perhaps dictated by the presence of regions A and D, since these regions contribute to the formation of the DH sites; alternatively, it might indicate a random assembly process.

The hsp70 gene also has the (CT)_h regions, one of which is located between the two proximal HSEs. This (CT)_h region has also been shown to interact with the CT-binding protein in vitro (Gilmour et al., 1989). A 52 bp cassette that contains the (CT)_h region bordered by two HSEs was shown to rescue heat-induced expression of the hsp26 gene deleted of its regulatory region, cP26ZAX, when the cassette was inserted at the deletion site (Simon & Lis, 1987). This cassette likewise reinstates a major DH site in these hsp26 transgenes (cP26Z-70a, cP26Z-70b: Fig. 2). Since HSEs appear to be occupied prior to heat shock in Drosophila, interaction of the hsp70 (CT)_h region with the CT-binding protein is a likely mechanism for dictating the formation of DH sites in the uninduced state. This hypothesis is consistent with the results of analyses of hsp26/lacZ transgenes with 5' deletions (Fig. 3) or

4. Discussion

Specific protein-DNA interactions can impose striking discontinuities in chromatin structure. These discontinuities are revealed as regions of DNA that are accessible to nucleases, such as DNase I. These DH sites have been shown to be free of nucleosomes and are often located in the promoter and regulatory regions of genes. The hsp26 gene has prominent DH sites that reside at defined positions in its regulatory region; here we have investigated the effect of various mutations in the hsp26 promoter/regulatory region on the formation of these DH sites. The chromatin structure was examined in transformed Drosophila lines bearing single inserts of variant hsp26/lacZ transgenes. In order to quantify the accessibility of the DNA in the DH sites, we measured the susceptibility of the DH sites to cleavage by a specific restriction enzyme. The (CT)_h region, which contains 32 bp of alternating C and T residues, appears to play a central and critical role in DH site formation (Table 1). In addition to the (CT)_h region, one of the other regions, A or D, is required for effective formation of the appropriate DH sites (Table 1).
with deletion or substitution of the (CT)_n region (Fig. 4).

Previous studies on the (CT)_n region (Glaser et al., 1990; Glaser & Lis, 1990) did not specify whether the (CT) sequence was involved in the formation of the DH sites or simply in transcriptional activation of hsp26. Our results here indicate that the (CT)_n sequence clearly has a role in DH site formation, but do not exclude the possibility of an additional role in transcriptional activation.

(b) The A and D regions contribute to the formation of the DH site

The boundaries of region A include the sequences from −351 to −135; this region contains HSE 6, an essential heat shock regulatory element, which lies in the distal DH site and is required for heat shock induction (Cohen & Meseelson, 1985; Pauli et al., 1986, Simon & Lis, 1987). HSF binds in vivo to this sequence after, but not before, heat shock (Thomas & Elgin, 1988). A short (7 bp) stretch of alternating C and T residues is observed at the proximal side of HSE 6; this could be a critical part of region A. Mutation of this distal (CT) sequence affects the transcriptional activity of the gene (Q.L., unpublished results). Region A also contains three sequence elements earlier designated HSE 3, 4 and 5, which are recognizable but poor matches to the HSE consensus sequence (Bienz & Polham, 1986; Lis et al., 1990). Genetic analysis indicates that these sequences do not significantly contribute to the heat shock response (Pauli et al., 1986; Simon & Lis, 1987; Glaser et al., 1990). Genomic footprinting has shown that in vivo HSE 3, 4 and 5 are packaged in a precisely positioned nucleosome; this packaging is not altered upon gene activation by heat shock (Thomas & Elgin, 1988).

Region D is located downstream from the transcription start site, and includes sequences from +11 to +632. Previous studies of heat shock genes have identified elements in this region that are necessary for efficient gene expression (see below); the present data indicate a significant role in the formation of the DH site. The transcriptional start of the hsp26 gene has been shown to be occupied by the RNA polymerase II complex before and after heat shock induction (Rougvie & Lis, 1990). In vitro footprinting analysis of the hsp26 promoter indicates binding of the TATA box-dependent complex to the region from −38 to +35; the sequences downstream from the TATA box are important in maintaining a stable complex (Dietz, 1990). The importance of this region in a related heat shock gene, hsp22, has been demonstrated. In this case, transcription is reduced to 1% to 10% of that of wild-type when a region from +1 to +26 is removed. When this region is left intact, up to 86% of the 5' untranslated sequence can be deleted without affecting transcription (Hultmark et al., 1986).

In one series of constructs studied here, upstream regions of the hsp26 gene (to +11) were fused to an hsp70/lacZ construct resulting in a chimeric gene containing hsp70 sequences from +89 to +260 (+250 is the translation start). These constructs lacking the hsp26 D region show a normal chromatin structure only with the intact −351 to +11 fragment of hsp26, when regions A and (CT)_n are present (Fig. 5). In contrast, only the (CT) region is needed for DH site formation in the presence of hsp26 region D (see Figs 2 and 4). The fact that the hsp70 sequence does not rescue DH site formation on the hsp26 promoter in the case of cP-135-70Z implies that the hsp70 sequences from +89 to +260 make no contribution to DH site formation in these constructs. Whether the functional element(s) of the D region lies in the sequence +11 to +89 and is missing, or whether the functional element(s) of the D region for the two genes are heterologous, remains to be determined. Nonetheless, the present findings clearly point to an important contribution to the chromatin structure of hsp26 promoter by region D.

How do regions A and D affect the formation of DH sites? As discussed above, these two regions contain many binding sites for regulatory proteins that are likely to influence the chromatin structure of the hsp26 promoter. These may act either directly through interactions with nucleosomes, or indirectly through promotion of assembly of the active transcription complex, to determine nucleosome position. Further analysis of hsp26 transgenes with small deletions within regions A and D is being initiated to define these effects more precisely.

(c) Possible molecular mechanisms for DH site formation

What is the mechanism behind the formation of stable DH sites by the A, (CT)_n and D regions? The TATA binding factor and RNA polymerase II are bound to the hsp26 promoter region in vivo prior to heat shock induction (Thomas & Elgin, 1988; Rougvie & Lis, 1990), initially suggesting to us that these proteins might be sufficient to establish the chromatin structure pattern. The prior binding of TFIIID has been shown to allow binding of the HSF to nucleosome templates in vitro (Taylor et al., 1991). However, this study demonstrates that effective DH site formation for hsp26 in vivo requires the (CT)_n·(GA)_n repeats, and at least one additional sequence element (region A or D) in addition to the promoter complex. The (CT)_n element might facilitate binding of TFIIID in vivo, allowing the transcription complex to compete successfully against nucleosomes in binding to this site. This hypothesis is supported by recent observations indicating a cooperative interaction between the TATA factor and the CT binding protein in vitro. Binding of the TATA factor stabilizes the interaction of the CT binding protein with the (CT)_n region of hsp26; conversely, the CT binding protein facilitates formation of the TATA box-dependent footprint on this promoter in vitro (Dietz, 1990). Proteins bound on regions A or D, together with the CT binding protein, might interact with each other to stabilize the
transcription complex, and therefore exclude nucleosomes from the hsp26 promoter. In the absence of the CT binding protein, nucleosomes or other non-histone chromosomal proteins may be distributed throughout the promoter region, causing the decrease in accessibility of the proximal DH site to DNase I and XbaI digestion that is observed. Nucleosomes have been shown to block the accessibility of a restriction enzyme to its recognition site within isolated nuclei (Fascher et al., 1990; Archer et al., 1991). In contrast, transcription factors, such as HSF, appear not to block the accessibility of a given restriction site within isolated nuclei (Jack et al., 1991; L.L.W., unpublished results). Thus, the results imply an alteration in the nucleosome array in the absence of the (CT)n sequence.

The ability of a specific transcription factor to facilitate the binding of TFIID has been shown in a mammalian system (Workman et al., 1990). In vitro reconstitution of a eukaryotic RNA polymerase II transcription system has shown a competition between binding of the polymerase complex and assembly of nucleosomes in the promoter region of many genes. Formation of a stable preinitiation complex is dependent on the binding of general transcription factor TFIID to the TATA element, a process that is itself blocked by prior nucleosome assembly (Matsum, 1987; Workman & Roeder, 1987; Losa & Brown, 1987; Knezevic et al., 1988). Once bound, however, TFIID can directly assemble a transcription complex even in the presence of histones (Becker et al., 1991). Proteins such as USF, a common upstream transcription factor, may function in part by facilitating the binding of TFIID, and hence favoring the formation of a stable transcription complex in preference to nucleosome binding at the promoter (Workman et al., 1990). In vitro these events can be manipulated by the order of addition of the components, whereas in vivo one would infer a general competition during the chromatin assembly that must follow DNA replication. Here the (CT)n element appears to play a critical role.

Additional examples in which nucleosomes may serve as negative regulators of gene expression come from studies on the Saccharomyces cerevisiae PH05 gene and on the MMTV promoter. In the case of the PH05 gene, the promoter is packaged in nucleosomes when the gene is repressed; these nucleosomes are displaced when the gene is active (Almer et al., 1986). Displacement or alteration of the nucleosomes appears to be a critical step in this gene activation (Straka & Hörz, 1991). At the MMTV promoter, a nucleosome masks a promoter proximal NF1 binding site when the promoter is inactive. Upon hormone induction, the binding of the glucocorticoid receptor to the MMTV promoter eliminates the nucleosome footprint and renders the NF1 site accessible (Cordingly et al., 1987; Richard-Toy & Hagar, 1987; Piña et al., 1990; Fascher et al., 1990; Archer et al., 1991). The preformed DH sites in the heat shock regulatory regions may be particularly critical, since HSF fails to bind to HSEs that are assembled into nucleosomes in vitro (Taylor et al., 1991).

From these studies and our work on the chromatin structure of the hsp26 gene, it appears that the chromatin structure of inducible promoters may fall into two classes. In cases such as those described above, the inactive state of a gene is correlated with the presence of nucleosomes along the promoter, blocking access to critical trans-acting factor binding sites (i.e. the TATA box and the NF1 binding site). Upon activation, these nucleosomes must be altered or displaced ("remodeled") in such a way as to allow access. Alternatively, in the case of hsp26 the chromatin structure of the promoter is specifically organized so as to allow access for the binding of the trans-acting factor, HSF; the "switch" is then controlled by a change in the binding affinity of HSF. Such a transition for the HSF in Drosophila has been demonstrated (Zimarino & Wu, 1987). This mechanism of activation allows for rapid switching. In fact, all genes reported to be associated with a "poised polymerase", including some constitutively expressed genes (Rougire & Lis, 1990), may fall into this category of having a "pre-set" accessible promoter.

Both mechanisms of gene activation require a highly defined chromatin structure as part of the regulatory apparatus. As shown here, the reduced ability to form a particular chromatin structure correlates with reduced inducible expression. Further mutagenic and biochemical analyses of the chromatin structure of other genes should lead to a better understanding of how these promoter structures are established and utilized.

We are grateful to the Elgin laboratory for critical reading of the manuscript. This work was supported by National Institutes of Health grant GM33778 to S.C.R.E., NIH grant GM22232 to J.T.L., National Service Awards GM14516 and GM07232 to L.L.W. and R.L.G., respectively, and a National Science Foundation Fellowship to B.D.A.

References


A stretch of "late" SV40 viral DNA about 400 bp long which includes the origin of replication is specifically exposed in SV40. *Cell*, 16, 453–466.


*Edited by P. von Hippel*