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DNase I Sensitive Domain of the Gene Coding for the Glycolytic Enzyme Glyceraldehyde-3-phosphate Dehydrogenase†

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ABSTRACT: We have cloned a 36-kilobase segment of chicken DNA containing the gene coding for glyceraldehyde-3-phosphate dehydrogenase [GAPDH (EC 1.2.1.12)], a glycolytic enzyme which is expressed constitutively in all cell types. Using defined segments of this cloned DNA as probes, we have determined the DNase I sensitive domain of the GAPDH natural gene in the hen oviduct. When nuclei isolated from hen oviduct were treated with DNase I under conditions known to preferentially degrade actively transcribed genes (i.e., 15–20% of the DNA rendered perchloric acid soluble), a region of approximately 12 kilobase(s) (kb) containing the GAPDH coding sequences and flanking DNA was found to be highly susceptible to digestion by DNase I. Approximately 4 kb downstream from the end of the coding sequences, there was an abrupt transition from the DNase I sensitive or "open" configuration to the resistant or "closed" configuration. The chromatin then remained in a closed conformation for at least 10 kb further downstream. On the 5′ side of the gene, the transition from a sensitive to a resistant configuration was located about 4 kb upstream from the gene. In addition, we have localized two repeated sequences in the area of DNA that was cloned. One of these is of the CR1 family of middle repetitive elements. It is located about 18 kb 3′ to the gene and as such lies well outside of the DNase I sensitive region which encompasses GAPDH. The other repetitive element is of an uncharacterized family. It is located upstream from the gene and appears to be within a region of transition from the DNase I sensitive to resistant states.

It is well documented that actively transcribed genes are maintained in chromatin in a configuration such that they are rendered relatively sensitive to degradation by DNase I [see Weisbrod (1982) for a review]. However, virtually all of the genes studied to date represent special cases in the sense that they either have been of viral origin (Panet & Cedar, 1977; Flint & Weintraub, 1977; Groudine et al., 1978), have been involved in highly specialized functions (Weintraub & Groudine, 1976; Garel & Axel, 1976; Miller et al., 1978; Wu et al., 1979; Bellard et al., 1980; Gerber-Huber et al., 1981), or have been present in multiple copies (Stalder et al., 1978; Samal et al., 1981). Furthermore, only in the case of the ovalbumin multigene family has an entire DNase I sensitive domain been defined (Lawson et al., 1980, 1982). Therefore, in order to gain further insight into the relationship between DNase I sensitivity and gene expression, we set out to clone a constitutive, "housekeeping" gene and compare its DNase I sensitive domain to that of ovalbumin, a hormonally regulated gene switched on in the chicken oviduct during differentiation of this tissue. Thus, we hoped to find common elements of gene expression even among two genes that presumably are regulated in a very different manner. For this purpose, we decided to clone the natural gene coding for the enzyme glyceraldehyde-3-phosphate dehydrogenase (GAPDH), a glycolytic enzyme expressed in all tissues. We chose this gene because its cDNA had already been cloned (Arnold et al., 1982; Dugaiczyk et al., 1983); thus, a probe was readily available.

The ovalbumin gene family DNase I sensitive domain has been defined by using solution hybridization as an assay to measure the concentration of specific DNA sequences remaining after extensive digestion of nuclei with DNase I (Lawson et al., 1980, 1982). It has been reported that this domain remains in the DNase I sensitive or "open" configuration for approximately 100 kilobase(s) (kb), encompasses the X and Y genes in addition to ovalbumin, and then makes a gradual transition to the resistant or "closed" conformation on each end (Lawson et al., 1980, 1982). In addition, several middle repetitive elements have been mapped within this domain (Stumph et al., 1981, 1982). Finally, since expression of the ovalbumin gene family is limited to the oviduct, these genes are found in a DNase I sensitive state in this tissue only (Garel & Axel, 1976; Lawson et al., 1980).

While the ovalbumin gene family exists in oviduct chromatin as a rather large DNase I sensitive domain with gradual transitions from sensitive to resistant states, other genes may exist in smaller, more compact domains. For example, it has been reported that in a cell line containing integrated adenovirus genes, the transition region from the open to the closed configuration lies in relatively close proximity to the ends of the transcriptional unit (Flint & Weintraub, 1977). Fur-

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thermore, the transition itself may occur over a region encompassing only a few nucleosomes (Flint & Weintraub, 1977).

In this study, we have defined the DNase I sensitive domain of GAPDH in the hen oviduct. Furthermore, we have searched this region of DNA for the repetitive elements characteristic of the ovalbumin domain. Finally, since the glycolytic enzymes are expressed in all cell types, we have examined the DNase I sensitivity of GAPDH in various tissues.

Materials and Methods

Cloning of DNA in the GAPDH Domain. All of the clones were isolated from a chicken DNA library obtained from Drs. J. D. Engel, J. B. Dodgson, R. Axel, and T. Maniatis. The library was prepared by inserting into the λ vector charon 4A chicken DNA that had been partially digested with *Hae*III and *Alu*I and then ligated to synthetic *Eco*RI linkers (Dodgson et al., 1979). The library was initially screened with a nearly full-length GAPDH cDNA clone provided by Dr. R. J. Schwartz (Dugaiczyk et al., 1983). Procedures for screening the library and isolating recombinant phage were as previously described (Colbert et al., 1980). The initial screening of the library with the cDNA probe yielded three overlapping clones, 233, 240, and 288, all of which contained the coding sequences for the GAPDH natural gene (see Figure 2). The clone 525 was isolated from the library by using the 3′-most *Eco*RI fragment from 288 as a probe. In a similar manner, 616 was isolated by probing the library with the 5′-most *Xba*I fragment of 233. Restriction mapping and confirmation of overlap by Southern analysis were according to established procedures (Colbert et al., 1980).

DNase I Sensitivity. Nuclei were purified from hen oviduct, liver, and spleen as previously described (Lawson et al., 1980). For treatment with DNase I, nuclei were suspended at a DNA concentration of approximately 1 mg/mL in buffer containing 10 mM tris(hydroxymethyl)aminomethane hydrochloride (Tris-HCl) (pH 7.4), 10 mM NaCl, 3 mM $MgCl_2$, and 0.35 M sucrose. Pancreatic DNase I (Worthington) was added to a final concentration of 40 μg/mL, and digestion was allowed to proceed at 37 °C until 15–20% of the DNA was rendered soluble in cold 7% perchloric acid. Purification of the DNA after in situ digestion was as previously described (Lawson et al., 1980). Control DNA was extracted from nuclei which had not been subjected to in situ nucleolysis. The high molecular weight DNA thus purified was subsequently sheared enzymatically to generate a fragment size distribution comparable to that obtained from nuclei digested with DNase I (Lawson et al., 1980). Hybridization probes were prepared from specific restriction fragments isolated from the various phage clones. Individual fragments were isolated by preparative electrophoresis in agarose gels followed by absorption to glass beads after dissolution of the appropriate gel slice in 6 M $NaClO_4$ (Vogelstein & Gillespie, 1979). DNA was eluted from the washed beads as described (Lawson et al., 1982). The DNA fragments were then labeled to high specific activity by nick translation with ^{32}P-labeled nucleotides (Amersham). Hybridizations were performed in sealed glass vials (Kontes) which in a final volume of 30 μL contained 10 mM *N*-(2-hydroxyethyl)piperazine-*N*′-2-ethanesulfonic acid (Hepes) (pH 7.0), 0.6 M NaCl, 2 mM ethylenediaminetetraacetic acid (EDTA), 7.5 mg/mL driver DNA (DNase I digested or randomly sheared control), and 500–800 cpm of probe. Samples were denatured at 100 °C for 10 min and then incubated at 68 °C for intervals ranging from 5 min to 24 h. Hybrid formation was determined by digestion with S1 nuclease (Miles Laboratories) in a standard manner (Lawson et al., 1980). Probe self-annealing was monitored in parallel vials containing 7.5 mg/mL yeast RNA. However, under the conditions employed, reannealing of the probe was never observed to occur above the background level of S1-resistant counts. Equivalent C_0t values have been plotted.

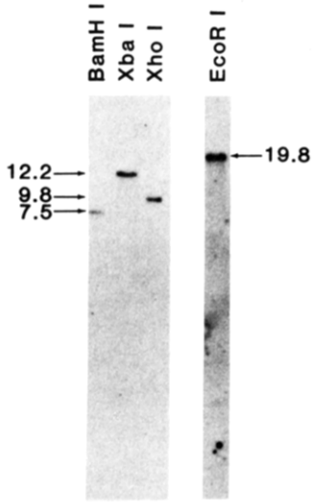

FIGURE 1: GAPDH is a single-copy gene in the chicken. High molecular weight DNA isolated from chicken liver was digested to completion with each of the restriction enzymes indicated. The fragments were then resolved on an agarose gel and blotted onto a nitrocellulose filter. The filter was hybridized to a GAPDH cDNA clone. Arrows indicate sizes of fragments in kilobases.

Detection of Repeated Sequences. Phage clones were digested with various restriction enzymes. The fragments were then resolved on agarose gels and transferred to nitrocellulose filters according to the procedures of Southern (1975). The filters were hybridized with a nick-translated probe consisting of total chicken genomic DNA. Procedures for hybridization and washing were as described (Lawson et al., 1982). Fragments hybridizing by this procedure were considered to be repetitive in the genome and were then checked to see if they would hybridize to a CR1-specific probe (Stumph et al., 1981).

Results

GAPDH Exists as a Single-Copy Gene in the Chicken. Since the success of this study was dependent upon gene walking, it was necessary to be certain that the GAPDH cDNA probe reacted with a unique sequence in the chicken genome. It is known, for example, that yeast contains multiple copies of GAPDH (Holland & Holland, 1980). Furthermore, isozymes of GAPDH have been reported to exist in other species (Lebherz & Rutter, 1967), a finding which could be indicative of the presence of multiple gene copies. However, as yet there have been no definite data reported in the literature concerning the status of GAPDH in the chicken genome. It has been demonstrated that cell-free translation of chicken embryo mRNA followed by immune precipitation and two-dimensional polyacrylamide gel electrophoresis of the precipitate yields only a single translation product (Macleod, 1981). Still this finding is only suggestive of the presence of a single gene copy. In addition, C_0t analysis has been used to demonstrate that in the chicken cell line MSB-1 GAPDH reanneals with unique sequence DNA (Kuo et al., 1982). However, this procedure was not sensitive enough to discriminate between one and several copies of the gene. Therefore, in order to prove conclusively that GAPDH is a unique gene in the chicken, high molecular weight DNA was digested with four different restriction enzymes; the fragments were resolved on an agarose gel and blotted onto nitrocellulose. The ensuing

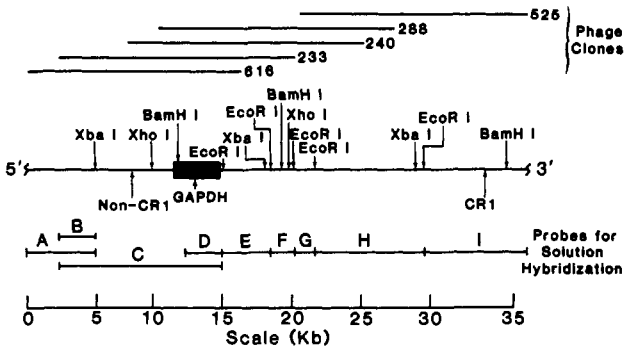

FIGURE 2: Cloning of GAPDH and its flanking sequences. All of the phage clones shown were isolated from a chicken library as described under Materials and Methods. Indicated below the map are the specific fragments used as probes for solution hybridization experiments. Repeated sequences were mapped and identified as described under Results.

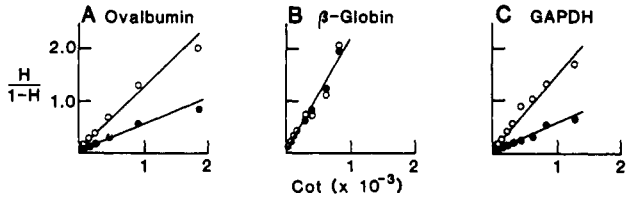

FIGURE 3: DNase I sensitivity of GAPDH. Panels A and B are control experiments demonstrating the difference in sensitivity between a nontranscribed gene, β-globin (panel B), and an actively transcribed gene, ovalbumin (panel A), in the hen oviduct. Panel C shows the same experiment with GAPDH. Probes used were double-stranded fragments of genomic clones for both globin and ovalbumin. A double-stranded fragment isolated from a cDNA clone was used as a probe for GAPDH. Closed circles represent driver DNA isolated from nuclei which had been digested by DNase I in situ. Open circles represent control driver DNA isolated in a high molecular weight form from hen oviduct and then sheared to the same average size as the DNase I digested driver DNA.

filter was hybridized to a ^{32}P-labeled GAPDH cDNA probe. The results presented in Figure 1 show that the probe reacts with only a single band in each case. Furthermore, the sizes of these bands are consistent with the data generated by restriction mapping of the clones isolated from the genomic library (Figure 2). Thus, we conclude that GAPDH exists as a single-copy gene in the chicken.

Restriction Map of GAPDH and Its Flanking Sequences. A partial restriction map of the GAPDH gene and its flanking sequences is presented in Figure 2. The overlapping phages isolated from the library are displayed in the upper portion of the figure. The specific fragments used as probes to establish the domain are shown below the map. The direction of transcription as indicated by the 5′ and 3′ labels in the figure was determined by Edward M. Stone (unpublished results). The coding sequences occupy an area of approximately 4 kb (Edward M. Stone, unpublished results).

GAPDH Is in the DNase I Sensitive Configuration in a Variety of Tissues. As has been reported by many workers, actively transcribed genes occur in chromatin in a form that leaves them relatively sensitive to degradation by DNase I, while genes which are not transcribed are resistant to digestion by this enzyme. Figure 3 depicts the results of an experiment designed to demonstrate this phenomenon and extend it to GAPDH as well. DNase I digested and control driver DNAs from hen oviduct were hybridized with probes containing portions of the genes of chicken ovalbumin (A), β-globin (B), and GAPDH (C) (Figure 3). The data have been transformed to a form such that when $H/(1-H)$ (where H represents the fraction of DNA hybridized) is plotted as a function of C_0t,

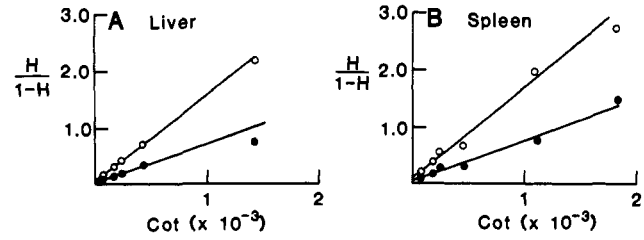

FIGURE 4: GAPDH gene exists in a DNase I sensitive configuration in various tissues. DNase I sensitivity was assayed as described under Materials and Methods by using fragment D (Figure 2), which contains the coding sequences, as the probe. Closed circles represent driver DNA from DNase I digested nuclei while open circles represent randomly sheared control DNA from liver (panel A) and spleen (panel B).

the concentration of tracer sequence in the driver preparation is proportional to the slope of the line obtained (Britten et al., 1974; Garel & Axel, 1976).

As has been reported, ovalbumin, which is expressed in the oviduct, is found in a DNase I sensitive configuration in this tissue (Garel & Axel, 1976). This can be measured by the slopes of the two lines which differ by a factor of about 3. Globin, on the other hand, which is not expressed in the oviduct, has been found to be resistant to digestion by DNase I in this tissue (Garel & Axel, 1976). This is confirmed in panel B of Figure 3 by the superimposibility of the two lines. By comparison with panels A and B, panel C demonstrates that the GAPDH gene is in a DNase I sensitive configuration in the hen oviduct. This is expected since the enzymes of the glycolytic pathway are expressed in this tissue.

Since the glycolytic enzymes are expressed in all tissues, one would expect GAPDH to be present everywhere in a DNase I sensitive conformation. In Figure 3, we show GAPDH to be DNase I sensitive in the hen oviduct. Also it has been reported that there is a DNase I hypersensitive region around the 5′ end of GAPDH in the chicken lymphoblastoid cell line MSB-1 (Kuo et al., 1982). However, we felt it necessary to extend these studies to other tissues as well. In Figure 4, data are presented which show that the GAPDH natural gene is in the "open" or DNase I sensitive configuration in both liver and spleen. In these experiments, fragment D (see Figure 2), which contains the coding sequences, was used as a probe to measure the relative concentrations in digested or control driver DNAs from both liver (A) and spleen (B). The results are clear, with the slopes of the curves differing by a factor of about 3 in each case.

DNase I Sensitive Domain of GAPDH in the Hen Oviduct. Figure 2 depicts the specific probes that were used to measure the DNase I sensitivity of the GAPDH gene and its flanking sequences. The probes labeled A–I cover the entire segment of DNA cloned and proceed in the 5′ to 3′ direction, with fragment D containing the coding sequences. As shown in Figure 5, fragments D and E, which contain respectively the natural gene and a 3.4-kb segment immediately adjacent to it, are clearly in a DNase I sensitive conformation. Proceeding in the 3′ direction, fragment F, which is a 1.7-kb segment of DNA next to fragment E, is obviously in a closed configuration. Fragments G and H, which are directly 3′ to fragment F and contain 1.5 and 7.9 kb of DNA, respectively, are also in the closed configuration. Fragment I, a 7.5-kb segment which is at the 3′-most end of the map, appears to be intermediate with regard to its DNase I sensitivity. Thus, it may lie in a transition region where the conformation of the chromatin switches from the closed to the open state.

As opposed to the abrupt transition from sensitivity to resistance observed on the 3′ side of the domain, the state of the

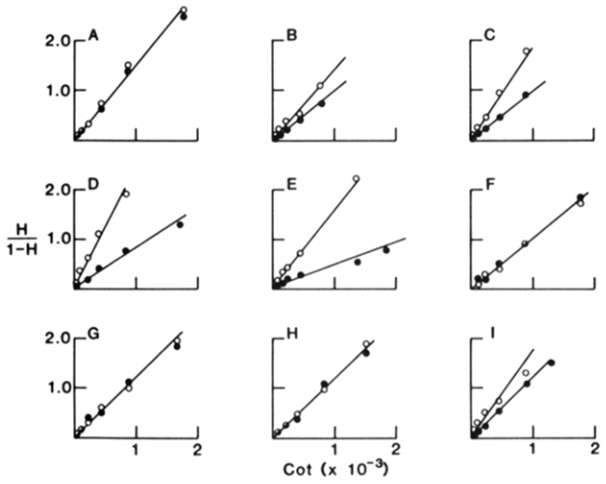

FIGURE 5: Determination of the GAPDH domain in the hen oviduct. Panel labels correspond to the probes indicated in Figure 2. Fragments A–I represent probes beginning at the 5′ end and ending at the 3′ end of the DNA cloned. Fragment D contains the coding sequences. Closed circles represent DNase I digested, and open circles represent control driver DNAs from hen oviduct.

chromatin on the 5′ side of the gene appears to change more gradually. Clearly, fragment A, the 5′-most segment, is in a closed configuration. Fragment B, the sequences of which are a subset of those in fragment A (see Figure 2), is nearly closed. In a similar manner, fragment C, which overlaps both fragment B and fragment D and contains the coding sequences for the gene, appears intermediate in its sensitivity, but with a ratio of slopes of only 1.8 compared to 3.0 for fragment D and 3.3 for fragment E, it is trending toward being closed. Probably a large portion of fragment C is in an open configuration. However, fragment B is clearly beginning to close down. Thus, we believe that the ratio of 1.8 for the entire C fragment represents an average between its sensitive 3′ end and its 5′ end which appears to become more resistant as one moves in this direction.

Repetitive Elements within the GAPDH Domain. The ovalbumin domain has been reported to contain middle repetitive elements of a specific family (CR1) which have been postulated to be involved in domain structure (Lawson et al., 1982; Stumph et al., 1983). Therefore, we thought it would be useful to search for repetitive sequences within the GAPDH domain as well. In this way, we hoped to find elements common to DNase I sensitive domains in general.

The DNA from clones 288, 240, and 233 (as indicated in Figure 2) was digested with *Eco*RI, electrophoresed on an agarose gel, and transferred to a nitrocellulose filter. The filter was then hybridized with nick-translated total chicken genomic DNA under conditions in which unique sequences fail to give a detectable signal (Shen & Maniatis, 1980). The autoradiograph is shown in Figure 6A. Neither 288 nor 240 yielded detectable signals even after prolonged exposure. However, the 11.7-kb *Eco*RI fragment (corresponding to fragment C in Figure 2) contained within 233 did give a signal and thus contains a repeated sequence. This repeat has been localized in fragment C as indicated in Figure 2. Finally, when this fragment was probed with nick-translated DNA containing the CR1 repeat, no signal was observed (Figure 6B). Thus, we conclude that this repeated sequence is not homologous to the CR1 family of middle repetitive elements which is found within the ovalbumin domain.

In a similar manner, when *Eco*RI-restricted DNA from clone 525 was probed with total genomic DNA, only the band corresponding to fragment I in Figure 2 gave a signal (data

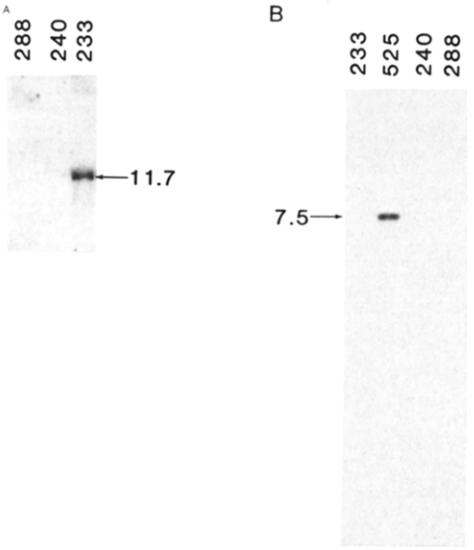

FIGURE 6: Mapping and identification of repetitive elements within the GAPDH domain. In panel A, the λ clones indicated were digested with *Eco*RI, resolved on an agarose gel, and blotted onto a nitrocellulose filter. The filter was then hybridized with nick-translated total genomic DNA. In panel B, the clones indicated were digested as in panel A but were hybridized to a probe containing the middle repetitive element CR1. The arrows indicate the size in kilobases of the fragments which hybridized.

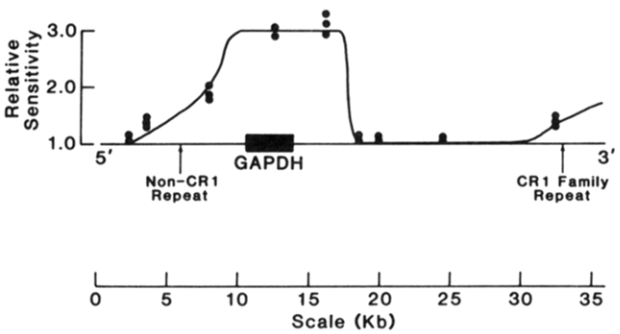

FIGURE 7: Summary of the GAPDH domain: schematic diagram illustrating the major findings of this study. Relative sensitivity is defined as the ratio of the slope of the line obtained with control driver DNA to the slope of the line generated by DNase I digested driver DNA. Closed circles represent the results of independent experiments.

not shown). However, as displayed in Figure 6B, when this phage was probed with CR1 DNA, the 7.5-kb fragment corresponding to fragment I (Figure 2) did hybridize.

Thus, we have discovered two repetitive elements flanking the GAPDH natural gene. A non-CR1 repeat has been located 5′ to the gene and appears to lie in a transition region where the configuration of the chromatin is changing from a DNase I sensitive to a resistant state. The other repeat is of the CR1 family and is located downstream from the gene. However, this repeated element seems to be located outside of the GAPDH domain and may be in a region where the chromatin undergoes a change from the resistant to the sensitive conformation.

Summary of the GAPDH Domain. Figure 7 summarizes the data we have obtained concerning the structure of the GAPDH domain. Due to both the limited resolution of the assay and the fact that we are probing relatively large segments of DNA in each experiment, we cannot precisely define the areas in which the configuration of the DNA shifts from one of sensitivity to one of resistance with regard to degradation by DNase I. However, it is apparent that the GAPDH gene is situated in a DNase I sensitive region approximately 12 kb

in length. This sensitive area is flanked on both sides by regions in which the chromatin undergoes a change in conformation from the DNase I sensitive to the resistant state.

Discussion

In this work, we have shown that in the hen oviduct the gene coding for the glycolytic enzyme glyceraldehyde-3-phosphate dehydrogenase exists in the form of a DNase I sensitive domain. This domain is about 15 kb in length and may contain only a single gene. The domain contains a central region about 10 kb long which is most sensitive to degradation by nuclease. This area is flanked on both sides by shorter regions in which the chromosomal configuration changes from a sensitive to a resistant state. On the 3′ side, this transition was abrupt and occurred over a distance of 1 kb or less. However, on the 5′ side, this transition appeared to be more gradual. However, due to both the limited resolution of the assay and the fact that our probes of the 5′ region are relatively large, we cannot precisely localize the area in which the conformation of the DNA shifts from sensitivity to resistance. Thus, it is possible that the 5′ transition region is as small as that found on the 3′ side.

Our main reason for undertaking this work was to have a DNase I sensitive gene domain to compare to that of ovalbumin, the only other eucaryotic gene studied definitively in this regard. By seeking common elements of domain structure between genes that are regulated in a very different manner, we hoped to gain further insight into the relationship between chromosomal architecture, as defined by nuclease sensitivity, and gene regulation.

While both the ovalbumin and GAPDH genes lie within DNase I sensitive domains, the precise details of each seem to vary. For example, while ovalbumin exists in a rather large domain encompassing a multigene family, GAPDH appears to exist as a single gene domain. Furthermore, while the ovalbumin domain makes gradual transitions on each end from DNase I sensitivity to resistance, the transition regions flanking GAPDH appear to be more abrupt. In addition, while the ovalbumin domain has a number of repeated sequences scattered throughout it, the GAPDH domain appears to have only one. Finally, using Southern blotting as a means of analysis, we have searched for direct homology between the ovalbumin domain and specific regions of the GAPDH domain. However, we have been unable to demonstrate any cross-hybridization (data not shown). In particular, the non-CR1 repeat located at the 5′ end of the GAPDH domain does not seem to be homologous to any of the repeated elements which map within the ovalbumin domain.

Yet there are similarities. For example, the GAPDH domain, like ovalbumin, contains a repetitive element located in the 5′ transition region. In addition, the 3′-most segment of DNA cloned appears to be in a transitional state with regard to DNase I sensitivity, changing from a closed to an open configuration. Interestingly enough, there is a CR1 family repeat located in this area. Perhaps in this region we are seeing the beginning of another domain. If this were the case, then it is possible that the presence of the repeat in this area is more than fortuitous. It might indicate that repetitive elements are required in at least one end of every domain in order to initiate or maintain its structure.

Since gene expression has been found to be correlated with DNase I sensitivity in every case that has been studied, it would appear that the alteration of nucleosome structure which leads to the formation of a DNase I sensitive domain is both a necessary and an early event in gene expression. However, formation of a DNase I sensitive domain is, by itself, an insufficient condition for transcription to occur. This is indicated by studies of globin in the erythrocyte (Weintraub & Groudine, 1976), ovalbumin in the oviduct (Lawson et al., 1982), and genes transcribed by pol III in the *Xenopus* oocyte (Coveney & Woodland, 1982) where the DNase I sensitive structure remained even after active transcription had ceased. Other events, such as the participation of regulatory molecules or the association of the genes with the nuclear matrix (Robinson et al., 1983; Ciejek et al., 1983), are undoubtedly required to maintain active transcription.

Thus, we conclude that DNase I sensitive domains provide structural capability for gene expression in eucaryotic cells. However, it seems that the precise structure of these domains varies with different genes and may be related to their mode of regulation. In the final analysis though, more gene domains need to be studied before we can draw general conclusions and define "consensus" DNase I sensitive domains for both constitutive and regulatable genes.

Acknowledgments

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Registry No. GAPDH, 9001-50-7; DNase I, 9003-98-9.

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Stability and Structure of Clathrin†

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ABSTRACT: The effects of urea on the dissociation and structural transitions of clathrin (8 S) have been evaluated by various techniques. The dissociation of the light chains in 3 M urea has been shown by light scattering, ultracentrifugation, and column chromatography. The dissociated components still retain the capacity to form the characteristic polygonal structure of the coat after removal of the urea. At higher concentrations of urea, the secondary and tertiary structures are eliminated, as documented by various spectroscopic techniques, i.e., tryptophan polarization and emission maxima, circular dichroism, and difference spectra. Two distinct transitions are observed by all techniques, one between 3 and 6 M urea and a second one which starts at 7 M but is still incomplete by 9.6 M urea. A concentration-dependent aggregation of clathrin chains occurs in 4 and 5 M urea solutions, as observed by light scattering and sedimentation. The results indicate that there are two large, independent domains in clathrin heavy chains and that each domain may have a single, highly cooperative transition.

Clathrin (8 S, $M_r \simeq 630\,000$) is the principal protein of the coat structure in both coated pits and coated vesicles (Pearse, 1975, 1976; Ungewickell & Branton, 1981; Pretorius et al., 1981). Clathrin dissociates from coated vesicles at pH values above ~7.5 (Pearse, 1978; Schook et al., 1979; Keen et al., 1979; Woodward & Roth, 1978, 1979). It is stable in this form to moderate changes in pH, ionic strength, and temperature (Pretorius et al., 1981). Clathrin can recombine with uncoated vesicles to form coated vesicles (Nandi et al., 1982a) or self-associate to form empty coats when the pH is reduced below ~7.0 (Pearse, 1978; Schook et al., 1979; Keen et al., 1979; Woodward & Roth, 1978, 1979; Nandi et al., 1980; Van Jaarsveld et al., 1981; Crowther & Pearse, 1981). The self-association is inhibited by salt (>0.2 M) whereas the reassociation to coated vesicles is not particularly sensitive to salt concentration (Nandi et al., 1982a).

Various aspects of the structure of native clathrin have been elucidated by electrophoresis in sodium dodecyl sulfate (SDS)[1] gels, electron microscopy, circular dichroism, and hydrodynamic methods. Purified preparations of clathrin contain essentially two distinctive species, i.e., three so-called heavy (M_r ~180 000) and three light (M_r 33 000) chains (Kirchhausen & Harrison, 1981; Ungewickell & Branton, 1981; Kirschhausen et al., 1983; Lisanti et al., 1982). The molecule has a symmetric structure, a triskelion, with three long arms (~450 Å) which have a bend in the middle (160–190 Å from the vertex) and a kink at their ends (Crowther & Pearse, 1981; Kirschhausen et al., 1983). Electron microscopic examination of the binding of antibodies to light chains or of ferritin-conjugated avidin to biotin-labeled light chains suggests that the three light chains are bound to the three heavy chains close to the vertex of the triskelion (Ungewickell et al., 1981; Kirschhausen et al., 1983). The light chains have been isolated by various methods, usually involving denaturation of the heavy chains or of both types of chains (Ungewickell et al., 1982; Lisanti et al., 1982). We do not know what role the light chains serve although clathrin cages can be re-formed without them from clathrin which has been digested with trypsin when in the form of cages (Schmid et al., 1982). Although the composition and structure of native clathrin have received considerable attention, the association of clathrin monomers to form triskelions and its interaction with light chains are awaiting investigation.

We have studied the effects of increasing concentrations of urea on clathrin in order to dissociate the subunits and to evaluate the structural transitions in the heavy chains of clathrin. The latter should afford information on the organization of the polypeptide chains in terms of its domains. Covalently bound substructures which fold independently are referred to as domains.

Materials and Methods

2-(*N*-Morpholino)ethanesulfonic acid (MES) and ethylene glycol bis(β-aminoethyl ether)-*N*,*N*,*N*′,*N*′-tetraacetic acid (EGTA) were from Sigma Chemicals. Analytical-grade urea

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[1] Abbreviations: MES, 2-(*N*-morpholino)ethanesulfonic acid; Gdn·HCl, guanidine hydrochloride (GdmCl in figures); EGTA, ethylene glycol bis(β-aminoethyl ether)-*N*,*N*,*N*′,*N*′-tetraacetic acid; SDS, sodium dodecyl sulfate; Tris, tris(hydroxymethyl)aminomethane.