Transcriptional and Translational Control of the Message for Transition Protein 1, a Major Chromosomal Protein of Mammalian Spermatids*  

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The spermatid transition proteins comprise a set of basic chromosomal proteins that appear during the period when spermatids are undergoing nuclear elongation and condensation, about midway between the end of meiosis and the release of spermatids from the seminiferous tubule. The transition proteins replace the histones but are themselves subsequently replaced by protamines, and they are not found in sperm nuclei. We have used a cDNA clone for the smallest transition protein (TP1, 54 amino acids) to show that its message first appears postmeiotically in late round spermatids. Thus production of TP1 is an example of haploid gene expression. The message remains translationally inactive for some 3–4 days before translation occurs in early elongating spermatids. While translationally repressed, TP1 message is nonpolysomal and has a discrete size of about 590 bases, including a 140 residue poly(A) tail. In contrast, polysome-associated message is of heterogeneous size due to variability of poly(A) lengths.

During mammalian spermatogenesis diploid spermatagonia divide mitotically several times to provide a population of spermatocytes that then proceed through meiosis to become haploid spermatids. Spermatid development involves a striking transformation of a morphologically unremarkable cell into the highly specialized spermatid (1, 2). One of the distinctive remodeling steps that occurs involves the change of nucleosomal chromatin to the highly condensed chromatin found in the sperm nucleus (3, 4). In mammals, early spermatids, which have nucleosomal chromatin, contain a mixture of somatic histones as well as testis-specific variants of H1 (5) and H2B (6) and sometimes H2A (7) and H3 (8, 9). About midway through spermatid development, the nucleus begins to condense, to elongate, and to become resistant to mechanical disruption (10, 11). These nuclear changes coincide with the replacement of both the somatic-type and testis-specific histones with a set of spermatid-specific chromosomal proteins now generally referred to as “transition proteins” (12, 13). Four transition proteins have been identified in the rat (12), and the situation is similar although not always identical in other mammals (13). Later still in spermatid development, the transition proteins are replaced by the distinctive cysteine-rich protamines found in the sperm nuclei of eutherian mammals (14–16).

RNA synthesis is not detected in spermatids once nuclear condensation begins (17–20). Accordingly, the production of messenger RNA for any proteins that appear after nuclear condensation must depend on RNA made at an earlier time. Indeed, as the pioneering studies of Dixon and colleagues (21, 22) have demonstrated, protamine message in the trout appears early in spermatogenesis (prior to meiosis) and is stored as a translationally inert RNA-protein particle until activation occurs late in spermatid development. Recently Hecht and associates (23) have examined this problem in mice and found that protamine message appears following meiosis in early haploid cells where it is translationally repressed until nuclear condensation is well under way. In mammals developing germ cells remain joined by cytoplasmic bridges (24), and this arrangement presumably mitigates or eliminates many, although not necessarily all (25), of the problems inherent to haploid gene expression.

TP1 was the first transition protein isolated (26), and is a 54-amino acid molecule notable for its high content of arginine and lysine and lack of an obvious globular region (27). It is present in many mammals including man (14, 26, 28). The recent isolation of a cDNA clone for TP1 permits analysis of TP1 message by molecular hybridization techniques. Based on the studies described below, we conclude that TP1 message first appears in late round spermatids but is not translated for some 3–4 days. Thus, like mouse protamine, TP1 message is transcribed in haploid cells and regulated at the translational level.

EXPERIMENTAL PROCEDURES

Materials—Sprague-Dawley rats were obtained from Harlan-Sprague-Dawley (Indianapolis, IN). For developmental studies, animals were from Building 207 of the Madison, WI facility. Plasmid pMH2 is a cDNA clone that contains the entire coding region for rat TP1. Plasmid pRD1 is a cDNA clone of rat β-actin that probably hybridizes to all classes of actin message and was kindly provided by P. Gunning (Veterans Administration Medical Center, Palo Alto, CA).

Isolation of RNA and Northern Blotting—Total high molecular weight RNA was isolated from tissues by proteinase K digestion and phenol/chloroform extraction, and precipitation from sodium acetate as described.

1 The abbreviations used are: TP1, transition protein 1. (Note that this protein has been designated simply TP previously. This change will bring the terminology in conformity with the other TP proteins [see Ref. 12].) HEPES, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid.

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described by Lee et al. (30). For standard Northern blots, RNA was denatured in a buffered mixture of formamide and formaldehyde and separated electrophoretically through agarose gels containing 2.2 M formaldehyde (31). It was transferred to nitrocellulose (BA85, Schleicher and Schuell) as described by Thomas (32). Prehybridization with the washing buffer was carried out as described by Maniatis et al. (33) for Southern blots with the final stringency wash in 75 mM NaCl, 15 mM Tris-HCl, 1 mM EDTA, 0.1% sodium dodecyl sulfate, pH 8.5 (0.5 X SET) at 65 °C for 20 min.

For Northern blots from polyacrylamide gels, RNA was denatured in 50% (v/v) formamide, 5 mM EDTA, pH 8, for 2.5 min at 100 °C. It was separated in a sequencing type gel (34) (12-cm long x 0.15-cm thick) containing 8 M urea and run at a voltage sufficient to maintain the surface temperature at about 50 °C. RNA was then transferred electrophoretically in a transblot cell (Bio-Rad) to a Zeta-Probe nylon membrane (Bio-Rad) according to the protocol provided by the supplier.

Preparation and Analysis of Polysomes—These procedures were based on those of Kleene et al. (23) with modifications (35, 36). Briefly, 1 g of decapsulated testis was homogenized in 4 ml of ice-cold 100 mM KCl, 10 mM MgCl₂, 20 mM HEPES, pH 7.5, 1 mg/ml heparin, 90 mg/ml cycloheximide with 10 strokes of a motor-driven type-B Teflon/glass homogenizer. The homogenate was centrifuged (5,000 × g) for 5 min to pellet intact cells and nuclei. The supernatant was transferred to 10 mg/ml dithiothreitol (Trition X-100) and centrifuged (12,000 × g) for 10 min. The supernatant (2 ml) was layered onto a 5-40% (w/v) sucrose gradient containing 100 mM KCl, 1 mM MgCl₂, and centrifuged for 3 h at 25,000 rpm in a Beckman SW-27 rotor at 4 °C. For control gradients in which polysomes were dissociated, EDTA was added to the 12,000 × g supernatant to make 100 mM. Gradients were fractionated using an ISCO (Lincoln, NE) 640 instrument and a UA-5 monitor. RNA was precipitated by adding 0.1 volume of 2 M sodium acetate and 2 volumes of ethanol. Precipitates were collected by centrifugation and dissolved in 1 ml of 0.1% sodium dodecyl sulfate, 10 mM Tris-HCl, 1 mM EDTA, pH 8, and digested with proteinase K (10 μg/ml). RNA was then extracted with phenol/chloroform and precipitated with ethanol from the phenol phase. For gradients of EDTA-released samples, 10 μg of carrier tRNA was added to each fraction prior to the initial ethanol precipitation. A sample of the RNA (1/50) isolated from each polysomal gradient fraction was incubated in 6% (v/v) formaldehyde, 1 mM NaCl, 50 mM sodium phosphate, pH 6.9, 5 mM EDTA for 15 min at 55 °C. The reaction was chilled on ice and applied as dots to BA85 nitrocellulose using a Scheicher and Schuell Minifold filtration device. The filter was then treated as for other Northern blots.

Digestion of RNA with RNase H—Selective digestion of poly(A) tracts was achieved by use of RNase H (37). The reaction mixture (50 μl) contained 20 μg of total high molecular weight RNA, 2 μg of oligo(dT)₁₅ (Research Biochemicals, Boston, MA), in distilled water, and was heated to 65 °C for 5 min. A 0.5% volume of 10X RNase H buffer was added (1X buffer contains 100 mM KCl, 20 mM Tris-HCl, pH 7.5, 10 mM MgCl₂, 50 μg/ml sucrose), and samples were incubated on ice for 15 min. RNase H was diluted with 6% (v/v) formaldehyde, 1 mM NaCl, 50 mM sodium phosphate, pH 6.9, 5 mM EDTA for 15 min at 55 °C.

In Vitro Protein Synthesis by Testis Tubules—Fresh adult testis was placed on ice immediately following dissection of the animal. The capsule was removed, and portions of tubules and interstitial tissue (0.5 g) were teased out using forceps. The tubules were placed in 25-ml Erlenmeyer flasks containing 3 ml of Krebs-Ringer buffered saline (38) (1.19 mM NaCl, 4.75 mM KCl, 16.1 mM NaHPO₄, 1.19 mM KHPO₄, 1.19 mM MgSO₄, 2 mg/ml glucose, adjusted to pH 7.4 with HCl) and kept on ice. Flasks were transferred to a shaking water bath at 34 °C and incubated in the presence of 10 μCi [³²P]methionine (Du Pont-New England Nuclear) for 2 h. Reactions were stopped by adding trichloroacetic acid to 4%. As a source of carrier TP1, the protein from 0.2 g of adult testis that is soluble in 4% but precipitated by 20% trichloroacetic acid was added. The contents were then homogenized with a Polytron (Brinkman Instruments) and worked up to select proteins that are not precipitated but precipitated by 20% trichloroacetic acid (26). Samples corresponding to 1/3 of the flask contents were separated electrophoretically in a 15% (w/v) polyacrylamide gel slab containing 0.9 μM acetic acid and 2.5 μM urea (39), and a fluorograph was prepared (40).

Miscellaneous—Radioactive probes were prepared from plasmid restriction fragments that were separated electrophoretically in polyacrylamide gels and recovered by electrolution in dialysis bags (41). Radioactive label was introduced by nick translation (42) using a [³²P]dideoxyribonucleotide from Du Pont-New England Nuclear and DNA polymerase I from Bacteriophage T4. For histological examination, testis samples were fixed in Carnoy’s solution, embedded in paraffin, sectioned, and stained with hematoxylin and eosin. Biochemicals were generally from Sigma. Measurements of pH were made at room temperature. Kodak X-Omat AR film was used for autoradiograms with Dupont Lightning Plus intensifying screens where appropriate. Whenever testis weight is specified, it refers to the combined weight of both testes from one animal.

RESULTS

TP1 appears in spermatid development during the period when nuclear condensation and elongation occur (12, 26), approximately stages 9-15 (49). Our initial goal was to determine whether TP1 message appears coincident with the protein or significantly earlier, perhaps even prior to the meiotic divisions. Mammalian spermatogenesis is a lengthy process, and about 9 days separate the second meiotic division from the beginning of nuclear condensation in spermatids (43). Thus, if TP1 message were to be transcribed premeiotically, it would have to appear at least 9 days before the synthesis of TP1 protein. In order to investigate this possibility, we decided to compare appearance of TP1 message and TP1 synthesis as the first cohorts of germ cells developed in young rats. Sensitive and unambiguous detection of message was made possible by the recent isolation of a cDNA clone for TP1.

TP1 message was determined by extracting total high molecular weight RNA and using the TP1 cDNA insert of pMH2 to identify specific sequences on Northern blots. To monitor TP1 synthesis, teased seminiferous tubules were incubated in vitro in the presence of labeled methionine. TP1 was extracted in 4% trichloroacetic acid. Following electrophoresis in acid urea gel slabs, the radioactive band was identified by fluorography and quantitated by excision and scintillation counting.

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In preliminary studies we found age alone to be an unreliable measure of the stage of sexual development of Sprague-Dawley rats but that testis weight was closely correlated with the appearance of TP1 message or protein. TP1 message is first detected in animals whose combined testes weighed slightly less than 0.5 g, corresponding in this case to an age of 22-24 days (Fig. 1). In contrast, TP1 synthesis by seminiferous tubules was first detected in testes whose combined weight was about 0.9 g, corresponding to animals about 26-27 days old (Fig. 2). When results from the two experiments are expressed quantitatively, and plotted to show appearance of message and protein as a function of increasing testes weight, it is clear that TP1 message appears distinctly before TP1 synthesis (Fig. 3). The lag between message appearance and protein synthesis is some 3-4 days based on correlation of testis weights and ages. Histological examination of testis samples taken over this stage of development shows that message is first detected when many tubules have substantial spermatids are now elongating at stages 9-13 (Fig. 4B). Since the lag between appearance of message and protein is much less...
than nine days, the message must be made postmeiotically in haploid cells. The fact that a significant lag does occur between synthesis and translation implies that the message is translationally repressed in round spermatids.

If TP1 message is translationally inactive for a period of days following the onset of its synthesis, then it might well be nonpolysomal in late round spermatids and so be exclusively nonpolysomal in developing tests that do not yet contain elongating spermatids. In adult tests, where all stages of spermatogenesis are present, we would expect that a significant fraction of TP1 message would be polysomal but that the portion originating from round spermatids would remain nonpolysomal. In order to examine this question, we prepared testicular polysomes from both immature and mature animals and separated them by sucrose gradient centrifugation to resolve nonribosomal material from small polysomes. RNA was isolated from fractions across the gradient, dotted on nitrocellulose, and then probed for either TP1 or actin (as a control). With adult tests we found about ¼ of TP1 message to be associated with small polysomes (Fig. 5A). EDTA treatment led to disruption of the adult polysomes, and all messages appeared at the top of the gradient (Fig. 5B). Actin message, which is not known to be under translational control, was associated for the most part with larger polysomes (Fig. 5A), which were also EDTA sensitive (Fig. 5, A and B). When polysomes were prepared from immature animals at the stage of development when TP1 message is present but translationally inactive, a quite different profile was obtained. In this case the message was recovered in the cytoplasmic fraction, but little or none of it was associated with polysomes (Fig. 6A). Instead, it all sedimented at the top of the gradient. The control probe demonstrated that actin message was largely polysomal in the immature animals, just as with the adults. EDTA treatment again caused both messages to sediment at the top of the gradients (Fig. 6B). Accordingly, from these experiments we conclude that TP1 message occurs in the
Fig. 4. Testis histology of young animals. Testes were fixed in Carnoy's solution, embedded in paraffin, sectioned, and stained with hematoxylin and eosin (× 160). A), material from testes of combined weight of 0.65 g. At this stage of development, TP1 message is readily detected, but TP1 synthesis is not. Most tubules contain round spermatids although those in the upper center and upper left contain pachytene spermatocytes as the most advanced cell type. B), material from testes of combined weight of 1.0 g. TP1 synthesis is detected in organs of this weight. The most advanced tubules contain elongating spermatids of steps 9-13.

cytoplasm of round spermatids in a translationally inert form, perhaps as some type of ribonucleoprotein particle. In the adult, when all stages of spermatogenesis are present and some cells are synthesizing TP1, a substantial fraction of TP1 message is polysomal.

It was of interest to see whether the polyadenylation state of TP1 message correlated with its association with active ribosomes. Poly(A) tracts can be removed selectively by digesting RNA with RNase H in the presence of oligo(dT) (37). Northern blotting can then be used to determine the size of the intact and deadenylated messages. We initially examined unFractionated RNA from immature animals since we had already shown that it occurs largely if not entirely in untranslating form. Untreated message migrated as a sharp band on a denaturing polyacrylamide gel with a size of about 590 nucleotides (Fig. 7, lane A). RNase H treatment reduced the

Fig. 5. Location of TP1 message in gradient-fractionated testis polysomes from an adult rat. Samples of a postmitochondrial supernatant were separated in a 5-40% exponential gradient of sucrose under standard conditions (A) or after adjusting each sample to 100 mM EDTA (B). RNA was prepared from each fraction and a sample spotted onto nitrocellulose and probed with either the insert for TP1 cDNA clone pMH2 or the insert from a cDNA clone for rat β-actin. At the top of each profile is shown the autoradiogram resulting from the dot hybridization of each fraction. Exposure time was 2 days for the actin filters and 9 days for the TP1 filters. The dots probed for TP1 were scanned with a densitometer, using film exposed in its linear response range. The data from these scans are plotted as a bar graph along with the absorbance of each gradient at 254 nm. The film density is in arbitrary units. The direction of sedimentation is from left to right.

Fig. 6. Location of TP1 message in gradient-fractionated testis polysomes from an immature rat. Details are the same as given in the legend to Fig. 5 except that polysomes were prepared from pooled testes in the weight range of 0.4-0.6 g/pair. At this stage of development TP1 message is present, but TP1 translation is not detectable. Densitometric scans of TP1 message shown as bar graphs are on a different scale than shown in Fig. 5.
message to a sharp band with a size of about 450 nucleotides (Fig. 7, lane B), indicating that the poly(A) stretch is uniform at about 140 bases. To examine translationally active message from adult animals, we fractionated a poly(A) gradient to separate the nonpolysomal and polysomal fractions of the message. The nonpolysomal message migrated as a tight band of about 590 nucleotides, just as found for the RNA from immature animals (Fig. 7, lane C). In contrast, the polysomal message migrated as a smear containing perhaps five subpopulations (Fig. 7, lane D). When treated with RNase H, the polysomal message collapsed into a tight band corresponding to the deadenylated message (Fig. 7, lane E). This indicates that the disperse size range of the untreated material was due to variable lengths of poly(A) rather than to random degradation. Since poly(A) lengths are known to grow shorter accompanying translation (45), this is not a surprising result. The fact that polysomal and nonpolysomal TP1 mRNA pools differ markedly with regard to polyadenylation argues that they are functionally distinct. The nonpolysomal mRNA is a distinct population that has not been exposed to the deadenylate activity that acts on the polysomal messages. Such protection from deadenylate might well be characteristic of sequestered, translationally repressed TP1 mRNA.

**DISCUSSION**

The availability of a cDNA clone for spermatid transition protein 1 has enabled us to determine whether the appearance of TP1 message is pre- or postmeiotic and whether it fore-shadows or is coincident with the appearance of TP1 protein. Because spermatogenesis is a long process, requiring some 48 days (43), we approached these questions by following the appearance of TP1 message and protein synthesis during normal sexual development in young rats. TP1 message was undetectable until about half the seminiferous tubules contained round spermatids. This must mean that no significant quantity of TP1 message is found in meiotic cells and that message accumulation does not occur until well after the meiotic divisions, in spermatids that have reached step 6 or 7. From our experiments we cannot conclude that the TP1 gene is totally inactive in spermatocytes or early spermatids, as the production of a highly unstable message precursor might go undetected. However, because of the striking accumulation of TP1 message that begins in late round spermatids, it is evident that the functional transcription of the gene occurs in haploid cells.

The issue of haploid gene expression has been of considerable interest. On the one hand, spermatids presumably require many new, stage-specific gene products. On the other, reliance on a haploid genome could lead to the phenotypic dominance of harmful recessive alleles. In fact, the picture now emerging indicates that haploid gene expression is apparently a common occurrence and perhaps the rule in mammals. Studies of this question prior to the availability of specific nucleic acid probes demonstrated that message-like RNA synthesis does occur actively in round spermatids (18, 20, 46, 47) and that poly(A) RNA isolated from purified round spermatids directs the synthesis of new proteins that are not encoded by translationally active RNA from spermatocytes (48, 49). When specific hybridization probes became available, it could be shown conclusively that messages for mouse protamines 1 and 2 (23, 50), mouse testis specific forms of α-tubulin (51) and actin (52), the testis specific form of phosphoglycerate kinase (PGK-2) (53), as well as other unidentified messages (50, 54), accumulate only in haploid cells. The situation is remarkably different for the protamine gene family in the trout, where the messages appear prior to the meiotic divisions even though they will not be translated until the end of spermatid development (21). Presumably the intercellular cytoplasmic bridges that join developing clones in the mammalian testis (24) make possible the diffusion of proteins or messages from cell to cell so that the spermatids are functionally diploid. A similar situation is found in Drosophila (55). Whether a functionally diploid state occurs for all gene products during spermatogenesis in these species is not known. Since certain genetic alleles are passed on poorly by male (though not by female) heterozygotes, it has been suggested that in some cases haploid gene expression during spermatogenesis does indeed lead to a haploid gamete phenotype (25).

Translational control is an increasingly well-documented method of regulating gene expression (56, 57). During spermatogenesis it is established for the control of protamine synthesis in both trout (21, 22) and mouse (23). In trout protamine messages appear prior to meiosis and are stored as cytoplasmic ribonucleoprotein particles until the final stages of spermatid development. High salt treatment recovers the protamine messages from these particles in translationally active form (22). In the mouse, protamine 1 message is similarly under translational regulation, although the period of control is shorter since the message in this case appears postmeiotically (23). Translational regulation is not established for other proteins used during spermatogenesis, but the nuclear transition proteins are a logical place to expect this since they appear just prior to the protamines in the program of nuclear transformation. We have presented two lines of evidence that support a translational mode of regulation of TP1 message in late round spermatids. First, there is a clear lag between the appearance of message and synthesis of TP1 in developing testes. Second, at a developmental stage when message is present but untranslated, it is found exclusively in the nonpolysomal compartment of the cell.

TP1 message was readily detected in developing testes in which only late round spermatids can be identified histologically. TP1 synthesis is not detected until elongating spermatids (step 9 onward) are present, and this result agrees with earlier estimates of the developmental appearance of TP1 (26) and with studies using separated cells that showed that elongated spermatids (but not round spermatids) incorporate la-
beled amino acids into TP1 (12, 58). Similarly, in the mouse the transition proteins are first detected in step 12 spermatids (59–61). The exact point at which TP1 is first made is difficult to determine. Spermatids pass through the early stages of nuclear elongation quickly, steps 9–11 requiring only about 1 day (43). As a result these cells are relatively scarce in the testis at any age and are difficult to obtain as highly purified populations (29, 62). Whether or not synthesis begins in these early elongating cells, it continues in cell populations enriched for steps 13–15 (12). Our developmental studies agree with these results, as TP1 synthesis was first observed in testes which, even in adult testes, occurs largely as a nonpolysomal message in round spermatids. In fact, the case for TP1 message is so strong that it originates from the stored message present in early spermatids in which the message is expressed. Since the length of time the message is expressed compared to the time it is stored is not known exactly, we cannot calculate precisely what proportion of the message ought to be polysomal and nonpolysomal in the adult testis, even if it was entirely polysomal in expressing cells. We may make an approximate calculation by assuming that message appears at the beginning of step 7 (late round spermatid) and that it is translated constantly from the beginning of step 9 until the end of step 14. As step 7 is long (3 days) while steps 9–11 are short (1 day altogether), this model has TP1 message present for 4 days in a repressed state and translated for 4 days. Therefore, we would expect only half of the message in the adult testis to be polysomal. This is rather close to the observed pattern. A shorter period of translation would obviously correlate with an even smaller proportion of polysomal message. It is unlikely that the nonpolysomal message from either adult or immature testes in an artificial product of polysome destruction during handling. RNAse H digestion showed that the message in the nonpolysomal region of the gradient has an intact and homogeneous poly(A) segment (~140 nucleotides), while the message from the polysomal region of the gradient has heterogeneous polyadenylation. Accordingly, the nonpolysomal message has been cycled minimally if at all through the polysomal population. A reasonable conclusion is that it originates from the stored message present in round spermatids. In fact, the case for TP1 message is very similar to that found for mouse protamine 1 message, which, even in adult testes, occurs largely as a nonpolysomal particle characterized by uniform polyadenylation. The polysomal message is in a minority and has distinctly shorter poly(A) tails (23).

Thus far, translational level control has been described only for nuclear proteins made late in spermatogenesis. It will be interesting to see what other proteins are subject to translational level control during spermatogenesis and to what extent the mechanisms of control are shared.

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REFERENCES

1. Bellve, A. R. (1979) in Oxford Review of Reproductive Biology (Finn, C. A., ed) Vol. 1, pp. 159–260, Oxford University Press, Oxford
2. Fawcett D. W. (1975) Dev. Biol. 44, 394–436
3. Kierszenbaum, A. L., and Tres, L. L. (1978) Fed. Proc. 37, 2512–2516
4. Loir, M., Bouvier, D., Fornells, M., Lanneau, M., and Subirana, J. A. (1985) Chromosoma (Berl.) 92, 304–312
5. Seyedin, S. M., and Kistler, W. S. (1980) J. Biol. Chem. 255, 5540–5544
6. Shires, A., Carpenter, M. P., and Chalkley, R. (1976) J. Biol. Chem. 251, 4156–4158
7. Trostle-Weige, P. K., Meistrich, M. L., Brock, W. A., Nishio, K., and Bremer, J. W. (1982) J. Biol. Chem. 257, 5560–5567
8. Trostle-Weige, P. K., Meistrich, M. L., Brock, W. A., and Nishio, K. (1984) J. Biol. Chem. 259, 8769–8776
9. Zweidler, A. (1984) in Histone Genes: Structure, Organization, and Regulation (Stein, G. S., Stein, J. L., and Marzulli, W. F., eds) pp. 339–371, John Wiley & Sons, New York
10. Grimes, S. R., Jr., Meistrich, M. L., Platz, R. D., and Hnilica, L. S. (1977) Exp. Cell Res. 110, 31–39
11. Loir, M., and Lanneau, M. (1978) Exp. Cell Res. 115, 231–243
12. Meistrich, M. L., Brock, W. A., Grimes, S. R., Jr., Platz, R. D., and Hnilica, L. S. (1978) Fed. Proc. 37, 2522–2525
13. Lanneau, M., and Loir, M. (1982) J. Reprod. Fertil. 65, 163–170
14. Coelingh, J. P., Monfoort, C. H., Rozijn, T. H., Gevers Leuven, J. A., Schipoff, R. O., Parve, E. P., Braunitzen, G., Schrank, E., and Rubfus, A. (1972) Biochim. Biophys. Acta 259, 1–14
15. Kleene, K. C., Distel, R. J., and Hecht, N. B. (1985) Biochemistry 24, 719–722
16. McKay, D. J., Renaux, B. S., and Dixon, G. H. (1985) Biochi. Rep. 4, 383–391
17. Menesi, V. (1985) Exp. Cell Res. 39, 197–224
18. Loir, M. (1972) Ann. Biol. Anim. Biochim. Biophys. 12, 203–219
19. Kierszenbaum, A. L., and Tres, L. L. (1974) J. Cell Biol. 60, 39–53
20. Geremia, R., Boitani, C., Conti, M., and Menesi, V. (1977) Cell Differ. 5, 345–355
21. Iatrou, K, Spira, A. W., and Dixon, G. H. (1978) Dev. Biol. 64, 82–98
22. Sinclair, G. D., and Dixon, G. H. (1982) Biochemistry 21, 1869–1877
23. Kleene, K. C., Distel, R. J., and Hecht, N. B. (1984) Dev. Biol. 105, 71–79
24. Dym, M., and Fawcett, D. W. (1971) Biol. Reprod. 4, 195–215
25. Erickson, R. P. (1978) Fed. Proc. 37, 2517–2521
26. Kistler, W. S., Geroch, M. E., and Williams-Ashman, H. G. (1973) J. Biol. Chem. 248, 4532–4543
27. Kistler, W. S., Noyes, C., Hsu, R., and Heinrikson, R. L (1975) J. Biol. Chem. 250, 1847–1853
28. Kistler, W. S., Geroch, M. E., and Williams-Ashman, H. G. (1975) Invest. Urol. 12, 346–350
29. Meistrich, M. L., Longtin, J., Brock, W. A., Grimes, S. R., and Mace, M. L. (1981) Biol. Reprod. 25, 1065–1077
30. Lee, D. C., McKnight, G. S., and Palmiter, R. D. (1978) J. Biol. Chem. 253, 3494–3497
31. Lehrach, H., Diamond, D., Wozney, J. N., and Boedtker, H. (1977) Biochemistry 16, 4743–4751
32. Thomas, P. S. (1980) Proc. Natl. Acad. Sci. U. S. A. 77, 5201–5205
33. Maniatis, T., Hardison, R. C., Lacy, E., Lauer, J., O’Connell, C., Quon, D., Sim, G. K., and Edelstiani, A. (1978) Cell 15, 687–701
34. Maxam, A., and Gilbert, W. (1980) Methods Enzymol. 65, 499–560.
35. Palacios, R., Palmer, R. D., and Schinke, R. T. (1979) J. Biol. Chem. 247, 2316–2321
36. Walden, W. E., Godefroy-Colburn, T., and Thach, R. E. (1981) J. Biol. Chem. 256, 11739–11746
37. Sippel, A. E., Stavrianopoulou, J. G., Schultz, G., and Feigelson, P. (1974) Proc. Natl. Acad. Sci. U. S. A. 71, 4635–4639
38. Benes, H. A. (1933) Z. Physiol. Chem. 124, 191–227
39. Panizzi, S., and Chalkley, R. (1969) Arch. Biochem. Biophys. 130, 337–346
40. Ostrowski, M. C., Kistler, M. K., and Kistler, W. S. (1982) Biochemistry 21, 3525–3529
41. McDonnell, M. W., Simon, M. N., and Studier, F. W. (1977) J. Mol. Biol. 110, 119–146
42. Maniatis, T., Jeffrey, A., and Kleid, D. G. (1975) Proc. Natl. Acad. Sci. U. S. A. 72, 1184–1188
43. Clermont, Y. (1972) Physiol. Rev. 52, 198–236
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44. Kistler, W. S., Keim, P. S., and Heinrikson, R. L. (1976) Biochim. Biophys. Acta 427, 752–757
45. Sheiness, D., and Darnell, J. E. (1973) Nature New Biol. 241, 265–268
46. Soderstrom, K.-O., and Parvinen, M. (1976) J. Cell Biol. 70, 239–246
47. Geremia, R., D'Agostino, A., and Monesi, V. (1978) Exp. Cell Res. 111, 23–30
48. Fujimoto, H., and Erickson, R. P. (1982) Biochem. Biophys. Res. Commun. 108, 1369–1375
49. Gold, B., Stern, L., Bradley, F. M., and Hecht, N. B. (1983) J. Exp. Zool. 225, 123–134.
50. Hecht, N. B., Bower, P. A., Waters, S. H., Yelicj, P. C., and Distel, R. J. (1986) Exp. Cell Res. 164, 183–190
51. Distel, R. J., Kleene, K. C., and Hecht, N. B. (1984) Science 224, 68–70
52. Waters, S. H., Distel, R. J., and Hecht, N. B. (1985) Mol. Cell. Biol. 5, 1649–1654
53. Erickson, R. P., Michelson, A. M., Rosenberg, M. P., Sanchez, E., and Orkin, S. H. (1985) Biosci. Rep. 5, 1087–1091
54. Dudley, K., Potter, J., Lyon, M. F., and Willison, K. R. (1984) Nucleic Acids Res. 12, 4281–4293
55. Lindsay, D. L., and Tokuyasu, K. T. (1980) in The Genetics and Biology of Drosophila (Ashburner, M., and Wright, T. R. F., eds) Vol. 2, pp. 225–294, Academic Press, New York
56. Lodish, H. F. (1976) Annu. Rev. Biochem. 45, 39–72
57. Austin, S. A., and Kay, J. E. (1982) Essays Biochem. 18, 79–119
58. Platz, R. D., Grimes, S. R., Meistrich, M. L., and Hnilica, L. S. (1975) J. Biol. Chem. 250, 5791–5800
59. Mayer, J. F., and Zirkin, B. R. (1979) J. Cell Biol. 81, 403–410
60. Mayer, J. F., Jr., Chang, T. S. K., and Zirkin, B. R. (1981) Biol. Reprod. 25, 1041–1051
61. Balhorn, R., Weston, S., Thomas, C., and Wyrobek, A. J. (1984) Exp. Cell Res. 150, 296–306
62. Ronnreil, L. J., Bellve, A. R., and Fawcett, D. W. (1976) Dev. Biol. 49, 119–131