Developmental and tissue-specific regulation of a novel transcription factor of the sea urchin

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We have identified a novel transcription factor that interacts with the promoter of four tissue-specific late histone H2A-2 and H2B-2 genes of the sea urchin by DNase I footprint, mobility shift, and methylation interference analyses. The binding site for this factor is required for efficient transcription of the H2B-2.1 gene both in vitro in nuclear extracts of gastrula embryos and in vivo in microinjected sea urchin embryos. This factor binds with equal affinity to the recognition sequences of all four histone genes in cross-competition assays. Moreover, the binding site of the H2B-2.2 promoter can functionally substitute for that of the H2B-2.1 gene in in vivo expression experiments. Nevertheless, all four binding sites share little sequence homology with each other. This transcription factor increases in abundance during embryogenesis and has been detected in the adult sea urchin only in the tube feet, where the late H2A-2 and H2B-2 genes are expressed specifically. Therefore, we refer to this factor as tissue-specific activator protein (TSAP). The close correlation between the presence of TSAP and the expression pattern of the late H2A-2 and H2B-2 genes suggests that this transcription factor is directly responsible for the developmental and tissue-specific regulation of these genes.

[*Key Words*: Tissue-specific transcription factor; late histone genes; in vitro transcription; sea urchin egg injection]

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Different gene families coding for the early, late, sperm, and cleavage-stage histones are expressed during the ontogeny of the sea urchin. The sperm histone genes are exclusively active during spermatogenesis, whereas the cleavage-stage histone genes are expressed in oogenesis. The highly repetitive early histone genes are also transcribed only during a short period of the sea urchin life cycle. These genes are activated upon meiotic maturation of the egg, are expressed maximally after fertilization during the rapid cleavage stages, and are already repressed in the hatching blastula embryo at a time when the transcripts of late histone genes start to accumulate rapidly [for review, see Maxson et al. 1983a; Busslinger et al. 1985].

The heterogeneous family of late histone genes consists of different single-copy genes that code for distinct histone H1, H2A, and H2B variants but identical H3 and H4 proteins (Childs et al. 1982; Maxson et al. 1983b; Busslinger and Barberis 1985; Kaumeyer and Weinberg 1986; Knowles et al. 1987; Cosson et al. 1988; Lai and Childs 1988). Late histone mRNAs are absent or barely detectable in the egg, start to accumulate in the blastula embryo, but reach their maximal levels only later in

embryogenesis (Maxson et al. 1983b; Knowles and Childs 1984; Busslinger and Barberis 1985). This developmental expression pattern is primarily the result of transcriptional activation rather than post-transcriptional regulation (Knowles and Childs 1984; Ito et al. 1988). Individual late histone genes differ, however, from each other in their precise temporal expression profile during late embryogenesis (Mohun et al. 1985; Kemler and Busslinger 1986). All late histone genes analyzed to date show the hallmarks of cell cycle-regulated histone genes, as they lack intron sequences and contain a 3'-terminal palindrome instead of a poly(A) addition site at their 3' end (for review, see Birnstiel et al. 1985). Hence, these genes appear to code for replication histones rather than replacement variants that predominate in differentiated, nondividing cells (Zweidler 1984). In support of this, late histone mRNAs could only be detected by in situ hybridization in mitotically active cells of late embryos (Angerer et al. 1985).

Late histone genes can be classified according to their expression pattern in the adult sea urchin. The somatic late histone genes are expressed in all adult tissues, whereas the expression of other late histone genes appears to be restricted to embryonic and larval development (Kemler and Busslinger 1986; Lieber et al. 1986; Halsell et al. 1987). The tissue-specific class of late histone genes so far consists of two nonallelic pairs of late H2A and H2B genes that we have isolated previously on

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two different cosmid clones from the sea urchin *Psammachinus miliaris* (Kemler and Busslinger 1986; Fig. 1). These two gene pairs code for different variants of the H2A-2 and H2B-2 subtypes and are differentially regulated during embryogenesis. The H2A-2.1 and H2B-2.1 genes of cosmid PmL1 reach their maximal expression level in the mesenchyme blastula embryo, whereas transcripts of the late H2A-2.2 and H2B-2.2 genes of cosmid PmL2 accumulate maximally only later in the pluteus larva (Fig. 1). We have previously interpreted this differential regulation as an indication that the two gene pairs may be expressed in different cell lineages of the late embryo (Kemler and Busslinger 1986). However, all four genes are subject to the same tissue-specific regulation in the adult sea urchin, as their transcripts could be detected only in the tube feet but not in any other tissue tested (Kemler and Busslinger 1986; Fig. 1).

Here, we report the identification of a novel transcription factor that interacts with each promoter of the tissue-specific H2A-2 and H2B-2 genes. This transcription factor increases in abundance during embryogenesis, is present only in tube feet of the adult sea urchin, and is therefore referred to as tissue-specific activator protein(s) (TSAP). The close correlation between the tissue distribution of TSAP and the expression pattern of the late H2A-2 and H2B-2 genes strongly suggests that this transcription factor is responsible for the developmental and tissue-specific regulation of these genes.

Results

A novel nuclear factor binds to the promoters of all tissue-specific late H2A-2 and H2B-2 genes

To identify transcription factors that may be responsible for the developmental and tissue-specific regulation of the *P. miliaris* late H2A-2 and H2B-2 genes (Fig. 1), we have analyzed their promoters by DNase I footprinting (Galas and Schmitz 1978) in nuclear extracts from gastrula embryos of the related sea urchin species *Paracentrotus lividus*. Several distinct regions of the four promoters are protected from DNase I digestion (Fig. 2). One of these DNA-binding activities footprints over the

octamer sequence ATTTGCAT present in both late H2B-2 promoters and therefore corresponds to the ubiquitous form of the octamer binding factor (OBF) that we have identified previously in embryonic extracts of the sea urchin (Barberis et al. 1987). Another nuclear factor protects the TATA box of the two late H2B-2 genes but fails to interact with the late H2A-2 promoters, suggesting that the H2B-2 TATA sequences have an intrinsically high affinity for TATA-binding proteins.

All other footprinting activities could not be identified at this time with known nuclear proteins. Among them is a factor that generates a DNase I footprint of similar extent on all four histone gene promoters. For reasons discussed below, we refer to this novel DNA-binding activity as TSAP. Competition with an excess of a double-stranded oligonucleotide containing the TSAP-binding site of the late H2B-2.2 promoter prevents DNase I protection of the TSAP-binding sites of all four promoters without affecting the interaction of other DNA-binding proteins (Fig. 2). These results indicated that either the same protein or closely related proteins with similar DNA-binding specificities interact with the promoter of all four tissue-specific histone genes. In a converse competition experiment, an oligonucleotide comprising the octamer motif abolishes only the interaction of the octamer-binding protein but not that of TSAP or the TATA-binding protein with the late H2B-2.1 promoter (Fig. 2). Hence, these nuclear factors can bind independently of each other under our DNase I footprint conditions. TSAP does not interact with the promoter of the early H2A, early H2B, sperm H2B, and somatic late H2B-1 gene in DNase I protection experiments with gastrula nuclear extracts (data not shown; see also Fig. 3). Therefore, we conclude that a characteristic feature of the promoters of tissue-specific late histone genes is the presence of a binding site for TSAP.

Nuclear factor TSAP binds with identical affinity to its recognition sequences in the late H2A-2 and H2B-2 gene promoters

We next examined whether the different TSAP-binding sites of the late H2A-2 and H2B-2 genes interact with

Figure 1. Genomic arrangement and expression pattern of the late H2A-2 and H2B-2 genes of the sea urchin *P. miliaris*. The H2A-2 and H2B-2 genes of cosmid PmL1 are coordinately regulated during embryogenesis, as are those linked on clone PmL2. The expression pattern of these two gene pairs is shown schematically. (+) Presence of the respective H2A-2 and H2B-2 mRNAs at different developmental stages and in adult tissues; (-) undetectable mRNA levels. For detailed expression profiles, see the original data of Kemler and Busslinger (1986).

Figure 2. DNase I protection of the late H2A-2 and H2B-2 gene promoters by nuclear extracts from sea urchin gastrula embryos. The coding strand of the late H2B-2.1 promoter, which was subcloned from position -130 (*PvuI*) to position +228 (*HindIII*), was 5'-end labeled at the *TaqI* site (+12), and that of the late H2B-2.2 promoter at the *HpaII* site (+75) in the gene. The noncoding strand of the late H2A-2.1 promoter, which was subcloned from position -200 (*PstI*) to position -18 (*HaeIII*), was 5'-end-labeled at the *XbaI* site in the polylinker of the pSP64 vector, and that of the late H2A-2.2 promoter at the *Clal* site, -135 upstream of the gene. These labeled DNAs were incubated in the gastrula nuclear extract prior to digestion with two different DNase I concentrations, as described in Materials and methods. The digestion products of the control reaction without protein (no XT) and of the footprinting reactions were analyzed together with the cleavage products of the A + G and C + T sequencing reaction on a denaturing 8% polyacrylamide gel. Double-stranded, ligated oligonucleotides, which either contained the octamer sequence ATTGCAT or the TSAP-binding site of the late H2B-2.2 gene, were used in a 1000-fold molar excess in competition experiments (for oligonucleotide sequences, see Materials and methods). DNase I-protected regions, together with the 5' end of the gene, are indicated to the right of each panel. OBF refers to the octamer-binding factor, TSAP to the tissue-specific activator protein, and TATA to the TATA-binding factor.

Figure 3. Identical affinity of TSAP for all four binding sites of the late H2A-2 and H2B-2 genes. (A) Electrophoretic mobility shift analysis. The cloned oligonucleotides containing the TSAP-binding sites of all four late H2A-2 and H2B-2 genes (see Materials and methods) were released from the polylinker of the vector pSP64 by *Ava*I and *Pst*I digestion prior to end labeling with [α - 32 P]dCTP and Klenow DNA polymerase. These labeled DNA probes were incubated in the gastrula nuclear extract, and protein–DNA complexes were resolved on a native 4% polyacrylamide gel, as described in Materials and methods. A double-stranded, multimerized oligonucleotide containing the TSAP-binding site of the H2B-2.2 gene (see Materials and methods) was used in a 600-fold molar excess as competitor DNA. (B1, B2) Specific protein–DNA complexes; (X) an unspecific complex; (F) free DNA. (B) Competition experiment. The TSAP competitor DNA (see above) and a 177-bp restriction fragment of the early H2B promoter (from position –102 to +75) were included in the binding reaction at the molar ratios indicated. Only the relevant part of the autoradiograph containing the two TSAP complexes, B1 and B2, are shown for each competition experiment.

the same DNA-binding protein(s) by electrophoretic mobility shift analysis (Fried and Crothers 1981; Garner and Rezvin 1981). Short end-labeled DNA fragments containing the four TSAP-binding sites were incubated separately with the gastrula nuclear extract, and specific

protein–DNA complexes were analyzed on a native polyacrylamide gel. All four TSAP-binding sites gave rise to three protein–DNA complexes (B1, B2, and X; Fig. 3A). Complex X is the result of an unspecific protein–DNA interaction, as its formation could not be prevented by competition with an excess of a double-stranded oligonucleotide containing the TSAP-binding site of the H2B-2.2 gene. However, the same oligonucleotide could compete specifically for B1 and B2, demonstrating that these two complexes correspond to the DNA-binding activity TSAP, identified previously in DNase I footprint experiments. The electrophoretic mobilities of both complexes are identical for three of the four DNA probes (Fig. 3A). The slightly faster migration of the complexes observed with the H2B-2.2 DNA is most likely due to the off-center position of the TSAP-binding site on this longer DNA fragment, which is indicative of protein-induced DNA bending (Wu and Crothers 1984). Therefore, we conclude that the TSAP-binding sites of all four genes generate the same two specific protein–DNA complexes in gastrula nuclear extracts.

The relative affinity of the proteins in complexes B1 and B2 for the four TSAP-binding sites was analyzed in competition experiments (Fig. 3B). Each of the four DNA probes was incubated separately with the gastrula nuclear extract in the presence of increasing amounts of the oligonucleotide containing the TSAP-binding site of the H2B-2.2 gene. The B1 complexes generated with all four DNA probes are competed away at identical competitor DNA concentrations. Likewise, the less abundant B2 complexes are all interfered with at the same, but lower, concentration of competitor DNA. Moreover, both complexes B1 and B2 that are detected by the same DNA probe appear to be competed at a similar molar ratio of protein to DNA, if their different abundance is taken into consideration. On the other hand, a 177-bp DNA fragment of the early H2B gene promoter does not affect the formation of these two complexes, even in a large excess, thus confirming the specificity of TSAP for the promoters of the tissue-specific class of late histone genes.

In summary, we have shown that each of the four TSAP-binding sites gives rise to the same two specific protein–DNA complexes and that these two complexes are generated at the same ratio relative to each other and exhibit an identical competition behavior in all four cases. Therefore, we conclude that TSAP binds with identical affinity to the promoters of all four tissue-specific late histone genes. The existence of two complexes suggests that TSAP either consists of closely related proteins with identical sequence specificity or corresponds to one and the same protein with different degrees of post-translational modification. It is also conceivable, however, that the two forms of TSAP differ from each other by the presence of an additional factor.

TSAP-binding sites differ considerably in primary sequence

The contact sites of TSAP within its four recognition

sequences were determined by methylation interference analysis (Siebenlist et al. 1980). End-labeled DNA fragments containing the TSAP-binding sites were partially methylated at G and A residues by dimethylsulfate (Maxam and Gilbert 1980) prior to their use in mobility shift experiments with gastrula nuclear extracts. Free and bound DNAs were eluted from the gel, cleaved at their modified purines, and analyzed on a denaturing sequencing gel. Methylated G and A residues that interfere with protein binding are expected to be underrepresented in the bound, relative to the free, DNA fraction.

The same methylated G and A residues interfere with protein binding in both the B1 and B2 complexes that are detected with the labeled H2B-2.1 DNA probe (Fig. 4). This evidence confirms that the two forms of TSAP exhibit identical DNA sequence recognition. The contact sites of TSAP on the H2A-2.1, H2A-2.2, and H2B-2.2 promoters were therefore only determined for complex B1 (Fig. 4). The results of these methylation interference experiments are summarized in Figure 4B, together with those of the DNase I footprint analysis (Fig. 2 and data not shown). TSAP generates an identical DNase I footprint pattern on all four binding sites, with 26 bp being protected on each DNA strand. However, comparison of these recognition sequences revealed little sequence similarity. In fact, only one nucleotide position within the deduced consensus sequence is completely conserved among all four binding sites. Nevertheless, the contact sites of TSAP correlate well with this consensus sequence. Whenever a methylated G or A residue in one sequence interferes with TSAP binding, a corresponding G or A residue at the same position in the other recognition sequences will also affect protein binding. It is interesting to note that opposite effects of G methylation are observed at three nucleotide positions, i.e., a methylated G residue interferes with TSAP binding in one sequence and enhances it in another sequence. Moreover, G residues that interfere with protein binding are present in two clusters to the left and right of the center of the DNase I-protected region and are separated on each DNA strand by 9–12 bp, i.e., by one turn of the DNA helix, on average. This suggests that TSAP interacts with its binding site only on one face of the DNA helix.

The octamer element and the TSAP-binding site are required for efficient in vitro transcription of the late H2B-2.1 gene

We mapped upstream promoter elements of the late H2B-2.1 gene by deletion mutagenesis and in vitro transcription. A series of deletion mutants extending to positions –760, –116, –76, and –53 upstream of the late H2B-2.1 gene was incubated together with the late H2A-2.1 control gene in the gastrula nuclear extract. In vitro-synthesized transcripts were mapped by S1 nuclease analysis with DNA probes that were unable to detect the endogenous late mRNAs present in the nuclear extract (see legend to Fig. 5). The transcription of both late genes is initiated in vitro correctly and efficiently (Fig. 5). Sequences far upstream were dispensable

in this transcription system, as removal of sequences from positions –760 to –116 did not affect the transcription efficiency of the late H2B-2.1 gene. Deletion of additional sequences to positions –76 and –53, which sequentially eliminated the octamer element and the TSAP-binding site, reduced the in vitro transcription rate stepwise by a factor of 2. A fourfold decrease of the transcription efficiency also is observed with an internal promoter deletion (from –149 to –53) that simultaneously removes both protein binding sites. Therefore, these results indicate that the recognition sequences for both the OBF and TSAP proteins are required for efficient in vitro transcription of the late H2B-2.1 gene in the gastrula nuclear extract.

TSAP is an essential transcription factor of the late H2B-2.1 gene in vivo

We have demonstrated previously that the *Psammechinus* H2B-2.1 gene is faithfully transcribed in microinjected embryos of the related sea urchin species *P. lividus* (Vitelli et al. 1988). This prompted us to study the role of the TSAP protein for transcriptional activation of the late H2B-2.1 gene in this in vivo expression system. Two substitution mutants were constructed in which the TSAP-binding site was replaced either by unrelated plasmid sequences or by the TSAP-binding site of the H2B-2.2 gene (Fig. 6). The wild-type H2B-2.1 gene, the two substitution mutants (containing 760 bp of 5'-flanking sequences), and a deletion mutant with only 53 bp of 5'-flanking sequences (including the TATA box) were coinjected with the late H2A-2.1 control gene into the cytoplasm of *Paracentrotus* eggs, as described by Vitelli et al. (1988). After fertilization, the injected eggs were allowed to develop to the swimming blastula stage, whereupon total RNA was extracted and assayed for the presence of exogenous H2A-2.1 and H2B-2.1 gene transcripts by SP6 mapping (Fig. 6).

Deletion of promoter sequences upstream of –53 caused a 13-fold reduction in H2B-2.1 gene transcription (Fig. 6, lanes 4 and 7), in agreement with the general observation that a TATA box is necessary but not sufficient for efficient expression of RNA polymerase II-transcribed genes in vivo. Substitution of the TSAP-binding site by unrelated plasmid sequences resulted in an 11-fold lower level of transcription, similar to that observed with the TATA box alone (lane 5). In contrast, the H2B-2.1 gene is equally well transcribed, if the TSAP-binding site of this gene is replaced by the homologous site of the H2B-2.2 promoter (lane 6). Footprint analysis of these mutants revealed that the plasmid substitution abolished TSAP binding without affecting the interaction of the octamer- and TATA-binding proteins, whereas exchange of the TSAP-binding sites did not alter the footprint pattern on the late H2B-2.1 promoter (data not shown). These results therefore demonstrate that TSAP is an essential transcription factor of the late H2B-2.1 gene and that different TSAP-binding sites can substitute functionally for one another in our in vivo expression system. These results, together with the in vitro transcription analysis of Figure 5, furthermore sug-

Figure 4. Contact sites of TSAP within the four recognition sequences of the late H2A-2 and H2B-2 promoters. (A) Methylation interference analysis. *HindIII*–*EcoRI* restriction fragments containing the cloned TSAP-binding sites of the four late H2A-2 and H2B-2 promoters (see Materials and methods) were 5'-end-labeled at either the *HindIII* or *EcoRI* site of the pSP64 polylinker and then used for methylation interference analysis of complexes B1 and B2 and unbound DNA (F) in the gastrula nuclear extract, as described in Materials and methods. Upper and lower strand and the indicated orientation (5' → 3') refer to the DNA sequences shown below. Methylated G and A residues that interfere with protein binding are indicated by large and small arrowheads, respectively. Strong and weak interferences are distinguished by solid and open arrowheads. The G > A reaction used to cleave methylated purines (see Materials and methods) invariably resulted in a lower efficiency of cleavage at methylated A residues, as compared to G residues. The signals of a few of the methylated A residues that interfered weakly with TSAP binding are indicated with open arrowheads and were only clearly visible in the free DNA lane (F) on longer autoradiographic exposures. (B) Summary of footprinting and methylation interference data. The relevant promoter sequences of the late H2A-2 and H2B-2 genes are shown (Kemler and Busslinger 1986). Numbers refer to nucleotide positions relative to the transcription initiation site. Brackets above and below the DNA sequences indicate the extent of DNase I protection by TSAP (Fig. 2 and A. Barberis, unpubl.). Arrowheads pointing toward the DNA sequence denote nucleotides where methyl-

ation interferes with TSAP binding; arrowheads facing away from the sequence indicate those positions where methylation enhances protein–DNA interaction. The table below the DNA sequences indicates the frequency with which the bases C, T, G, and A occur at the different positions within the upper strand of the four TSAP-binding sites. The C residue at the only invariant position is boxed. Those positions where only two different nucleotides were observed are highlighted by boldface numbers and were used to deduce the consensus sequence (bottom).

Figure 5. Analysis of deletion mutants of the late H2B-2.1 promoter by in vitro transcription. The DNA templates A–E were generated by cloning late H2B-2.1 sequences from position +228 (in the gene) to the 5' endpoint indicated into the *Hind*III site of the pSP64 vector. A *Bam*HI fragment from –149 to –53 was deleted in template E. The late H2A-2.1 template contained sequences from +210 to –6500 in the *Hind*III site of the pSP64 vector. The promoter deletion mutants of the H2B-2.1 gene were transcribed together with the H2A-2.1 control gene in the gastrula nuclear in the presence of all four unlabeled ribonucleotide triphosphates. In vitro transcripts were assayed by S1 nuclease analysis with H2B-2.1 and H2A-2.1 DNA probes that were 5'-end-labeled at the *Xba*I site in the downstream pSP64 polylinker and extended into the 5'-flanking sequences up to –760 and –200, respectively. These DNA probes were unable to detect the endogenous late mRNAs present in the extract, as they were not labeled in the gene sequences but in the adjacent polylinker. The relative intensity of the S1 signals was measured by densitometric scanning of the autoradiographs. The binding sites for OBF, TSAP, and the TATA-binding factor are indicated.

gest that TSAP is required to mediate the transcriptional effect of the octamer-binding protein onto the H2B-2.1 promoter.

The level of TSAP correlates with the expression pattern of the late H2A-2 and H2B-2 genes during embryogenesis and in the adult sea urchin

So far, our analysis suggests that the transcription factor TSAP may be a major determinant of the developmental and tissue-specific regulation of the family of late H2A-2 and H2B-2 genes. To test this hypothesis, we have compared the expression level of the late H2B-2.1 gene to that of the TSAP protein at different developmental stages and in adult tissues. The same amount of total RNA (30 μ g, except for 10 μ g of gastrula and pluteus RNA) was used to titrate transcripts of the late H2B-2.1 gene by S1 mapping with a DNA probe that was 5'-end-labeled at a gene-internal restriction site and extended into the 5'-flanking sequences. Only the relevant part of

Figure 6. Functional equivalence of TSAP-binding sites for in vivo transcription of the late H2B-2.1 gene in microinjected sea urchin embryos. The TSAP-binding site of the late H2B-2.1 gene (template A) is flanked on both sides by *Sac*II and *Bam*HI restriction sites (see Fig. 4). The wild-type 19-bp sequence between these sites was replaced in template F by the pSP64 polylinker sequence ACCTGCAGGTC and in template G by the oligonucleotide TACGCAACGAAGCGAAGAC containing the TSAP-binding site of the H2B-2.2 gene. The entire H2B-2.1 gene with 40 bp of 3'-flanking sequences and 760 bp (A,F,G) or 53 bp (D) of 5'-flanking sequences was present on these templates, whereas the late H2A-2.1 control gene contained 200 bp and 5 kb of 5'- and 3'-flanking sequences, respectively. All plasmids were linearized at the *Pvu*II site in the vector pSP64. These *P. miliaris* H2A-2.1 and H2B-2.1 gene constructs were coinjected into the cytoplasm of unfertilized eggs of the sea urchin *P. lividus* at DNA concentrations of 60 ng/ μ l and 30 ng/ μ l, respectively. After fertilization, the injected eggs were allowed to develop to the swimming blastula stage, whereupon total RNA was isolated and analyzed by SP6 mapping, as described in Materials and methods. RNA from *Psammechinus* gastrula embryos was used in lanes 1 and 2 as a control for the SP6 mapping of late H2B-2.1 and H2A-2.1 gene transcripts, respectively. Correctly initiated H2B-2.1 gene transcripts generate a doublet of RNase-resistant bands in lanes 1, 4, 5, 6, and 7, which is most probably an artifact of SP6 mapping with this particular late H2B-2.1 probe. No cross-hybridization was detected between endogenous *Paracentrotus* transcripts and the *Psammechinus* SP6 RNA probes (lane 3).

the autoradiograph that contains the S1-resistant DNA fragment corresponding to the correct 5' end of the late H2B-2.1 mRNA is shown in Figure 7. Consistent with previous studies (Busslinger and Barberis 1985; Kemler and Busslinger 1986), this mRNA is abundant in gastrula and pluteus embryos, is present at similar but lower amounts in blastula embryos and in tube feet, and is un-

Figure 7. Correlation between late H2B-2.1 gene expression and the distribution of TSAP in sea urchin embryos and adult tissues. (Top) S1 nuclease analysis. Ten micrograms of total gastrula and pluteus RNA and 30 μ g of RNA from blastula embryos, coelomocytes, tube feet, ovaries, and testes were analyzed by S1 mapping with a late H2B-2.1 DNA probe that was labeled at the *Hinf*I site at position +145 in the gene and extended up to the *Bam*HI site at -55. Only the relevant part of the autoradiograph with the S1-resistant DNA fragments corresponding to the correct 5' end of the late H2B-2.1 mRNA is shown. (Bottom) Electrophoretic mobility shift analysis. End-labeled DNA fragments containing the TSAP-binding site (Materials and methods) or the octamer element of the immunoglobulin heavy-chain gene enhancer (Gerster et al. 1987) were used as specific probes for TSAP and OBF in mobility shift experiments with nuclear extracts from the same tissues analyzed above by S1 mapping. For each extract, the same amount of nuclear protein was analyzed with both probes. Only the relevant protein-DNA complexes are shown. A 600-fold molar excess of the TSAP and octamer competitor oligonucleotides (Materials and methods) was used for competition in the tube foot nuclear extract.

detectable in testis, ovary, and coelomocytes. Nuclear extracts from each of these tissues were then prepared and tested, in mobility shift experiments, for their integrity with a labeled octamer oligonucleotide and for the presence of TSAP with a late H2B-2.1 DNA probe. Again, the signals of only the relevant protein-DNA complexes are shown in Figure 7. The octamer-binding protein is present in comparable amounts in all extracts tested, in agreement with our previous analysis (Barberis et al. 1987). The same two forms of TSAP are detected in all embryonic extracts. Interestingly, only the more slowly migrating form increases in abundance during embryogenesis. Our most important observation is, however, that the TSAP-specific DNA probe detects a similar protein-DNA complex also in nuclear extracts of tube feet but not in extracts of any other adult tissue tested. This DNA-binding activity of the tube foot extract was positively identified as TSAP, as it could only be competed with the TSAP-binding site, but not with an octamer oligonucleotide. Taken together, these data demonstrate that TSAP is most abundant in gastrula and pluteus embryos, is present in smaller amounts in blas-

tula embryos and tube feet, and is undetectable in testis, ovary, and coelomocytes. Hence, the concentration and tissue distribution of the TSAP protein correlate well with the expression level of the late H2B-2.1 gene during embryogenesis and in the adult sea urchin, suggesting that this transcription factor is directly responsible for the developmental and tissue-specific regulation of the late H2A-2 and H2B-2 genes.

Discussion

Previously, we have characterized two pairs of nonallelic late histone H2A-2 and H2B-2 genes that are expressed in a tissue-specific manner in the adult sea urchin (Kemler and Busslinger 1986). Now we have identified a nuclear factor that binds to each promoter of this class of late histone genes and that is present only in tissues where these genes are expressed. As shown by mutagenesis of its binding site, transcription of the late H2B-2.1 gene is dependent on this factor both in vitro in gastrula nuclear extracts and in vivo in microinjected sea urchin embryos. For these reasons we refer to this novel transcription factor as TSAP. TSAP neither interacts with the promoters of the sperm H2B, early H2A, and H2B genes nor with a somatic late H2B-1 gene (Cosson et al. 1988). Hence, this transcription factor appears to be specific for the tissue-specific class of late histone genes.

Unusual promoter structure of the late H2B-2 genes

The promoters of all vertebrate and sea urchin H2B genes analyzed to date are characterized by the juxtaposition of the octamer sequence ATTTGCAT next to the TATA box (Harvey et al. 1982; Perry et al. 1985; Sive et al. 1986; Barberis et al. 1987; Maxson et al. 1987). The octamer element in this position recently was shown to confer cell-cycle-specific regulation to the promoter of a human H2B gene (LaBella et al. 1988), presumably through the interaction of the ubiquitous OBF with the TATA-binding protein (Fletcher et al. 1987). These experiments and the evolutionary conservation therefore would suggest that the close proximity of the octamer sequence and the TATA box is an obligatory feature of cell-cycle-regulated H2B genes. Both late H2B-2 genes of the sea urchin also appear to belong to the replication-dependent histone genes, as they lack intron sequences and contain the characteristic 3'-terminal palindrome at their 3' end (Kemler and Busslinger 1986). However, their promoter structure is an exception to the rule, as the binding site for the tissue-specific transcription factor TSAP is immediately adjacent to the TATA box, whereas the octamer element is located farther upstream at position -100. Both of these sequences were shown to be essential promoter elements of the late H2B-2.1 gene in in vitro transcription assays. Furthermore, in vivo expression experiments revealed that the TSAP-binding site is the major determinant of the H2B-2.1 promoter activity, suggesting that TSAP mediates the effect of the octamer-binding protein onto the transcription machinery.

TSAP recognizes motifs of considerable primary sequence variation

Mobility shift experiments revealed two different forms (B1, B2) of TSAP in nuclear extracts of sea urchin embryos. A potential third form with intermediate electrophoretic mobility was detected in the tube foot extract. However, it may correspond to complex B1 of the embryonic extracts, as even the ubiquitous OBF of the tube foot extract migrates for unknown reasons slightly faster than the respective protein of the embryonic extracts (Fig. 7). The two embryonic forms of TSAP contact the same nucleotide positions within the recognition sequence and bind with identical affinity to all four sites of the late H2A-2 and H2B-2 genes. It is therefore likely that these two forms differ from each other by post-translational modification of the same protein or by the presence of an additional factor. Alternatively, TSAP may consist of a family of related proteins with identical sequence recognition but different functions in analogy to the octamer-binding proteins (Staudt et al. 1986, 1988; Barberis et al. 1987; Fletcher et al. 1987; Gerster et al. 1987; Scheidereit et al. 1987). However, we consider it unlikely that the faster migrating form (B2) in the embryonic extracts is a proteolytic degradation product, as the same ratio of the two forms of TSAP was observed in several independent extract preparations.

Previous sequence comparisons revealed little homology between the promoters of the late H2A-2 and H2B-2 genes (Kemler and Busslinger 1986). Therefore, it was surprising to see that the same DNA-binding activity interacts with the promoter of each of these genes. TSAP generates an identical DNase I footprint with all four recognition sequences and binds them with the same affinity as shown by cross-competition experiments. These TSAP-binding sites also are functionally equivalent, as the site of the H2B-2.1 gene can be replaced by that of the H2B-2.2 promoter without affecting the transcription efficiency of this gene in microinjected sea urchin embryos. Despite all of this evidence, only a poor consensus sequence could be deduced, even when comparing the four TSAP recognition sequences in all different combinations of their orientation. However, the contact points of TSAP within the four binding sites correlate quite well with this consensus sequence and map to two clusters of G residues that are separated on each DNA strand by about one turn of the DNA helix. Therefore TSAP appears to interact with its recognition sequence only on one face of the DNA helix. Our most important conclusion, however, is that TSAP-binding sites can tolerate a considerable degree of primary sequence variability. Hence, we assume that other parameters, such as the local DNA conformation, may also contribute critically to the specificity of the TSAP–DNA interaction. A recent crystallographic analysis of the *trp* repressor–operator complex has indeed revealed that this *Escherichia coli* protein contacts the phosphate groups directly, but not the bases of the DNA, via hydrogen bonds, and that the operator sequence is only indirectly recognized through its effect on the conformation of the phosphate backbone (Otwinski et al.

1988). A prototype of a eukaryotic transcription factor with a seemingly degenerate mode of sequence recognition is the yeast zinc finger protein HAP1. This protein is involved in the coordinate regulation of the *CYC1* and *CYC7* genes by binding with equal affinity to upstream activating sites (UAS) of no obvious sequence similarity (Pfeifer et al. 1987). Other examples are the vertebrate enhancer-binding proteins C/EBP (Landschulz et al. 1988) and TEF-1 (Davidson et al. 1988), both of which interact with two unrelated sequence motifs.

Developmental and tissue-specific regulation of TSAP

Expression of the late H2A-2 and H2B-2 genes has been observed exclusively in tube feet among all the tissues tested of the adult sea urchin (Kemler and Busslinger 1986). The presence of the transcription factor TSAP correlates strictly with this expression pattern, as it too was only found in nuclear extracts of tube feet but not in other adult tissues. Late H2A-2 and H2B-2 mRNAs are not detectable in the ovary and egg but accumulate after fertilization during early development (Busslinger and Barberis 1985). TSAP is also absent in mature ovaries but is present in early embryos. It increases in abundance from the blastula to the gastrula and pluteus stage and hence parallels the expression pattern of the late H2A-2.1 and H2B-2.1 genes during this period of development. All of this evidence suggests strongly that TSAP is directly responsible for the developmental and tissue-specific regulation of the late H2A-2 and H2B-2 genes. Indeed, substitution of the TSAP-binding site by unrelated sequences drastically reduced the expression of the late H2B-2.1 gene in microinjected embryos. However, experiments with transgenic sea urchins (Flytzanis et al. 1985) will be required ultimately to prove a causal relationship between TSAP and the specific regulation of this class of late histone genes.

How can the transcription factor TSAP be responsible for the developmental regulation of both H2A-2 and H2B-2 gene pairs, although they are regulated differentially during embryogenesis (Kemler and Busslinger 1986)? Indeed, the level of TSAP at different embryonic stages correlates strictly only with the expression pattern of the late H2A-2.1 and H2B-2.1 genes that reach their maximal expression in the mesenchyme blastula embryo long before the maximal accumulation of the late H2A-2.2 and H2B-2.2 mRNAs in the pluteus larva (see Fig. 1). However, mobility shift experiments have failed to reveal a pluteus-specific form of TSAP or of any other transcription factor that interacts with the H2A-2.2 and H2B-2.2 promoters. Furthermore, the TSAP-binding site of the H2B-2.2 gene can functionally substitute for that of the H2B-2.1 gene in microinjected blastula embryos. Therefore we assume that the same TSAP is also involved in the transcriptional activation of the late H2A-2.2 and H2B-2.2 genes. Although TSAP appears to be necessary for the developmental regulation of these genes, it clearly cannot be sufficient. At least one additional regulatory step must act at the level of the chromatin domain containing these two genes by controlling the accessibility of their promoters for tran-

scription factors such as TSAP. One possible interpretation of the differential regulation of H2A-2 and H2B-2 genes during embryogenesis is that these two gene pairs may be expressed in different cell lineages of the late embryo (Kemler and Busslinger 1986). Given the tissue-specific distribution of TSAP in the adult sea urchin, it is not unreasonable to assume that this transcription factor may also be present only in certain cell lineages of the late embryo, including those that express the late H2A-2 and H2B-2 genes.

The cell-type-specific expression of eukaryotic genes is regulated primarily at the transcriptional level (for review, see Darnell 1982), presumably through the interaction of cell-type-specific transcription factors with enhancer and promoter sequences. Recently, it has been shown that the B-cell-specific transcription of immunoglobulin genes is determined by a lymphoid-specific octamer-binding protein (Staudt et al. 1986, 1988; Wirth et al. 1987; Müller et al. 1988) and that the erythrocyte-specific regulation of globin genes depends, at least in part, on the erythroid-specific factor NF-E1 (Evans et al. 1988; Galson and Housman 1988; Reitman and Felsenfeld 1988; Superti-Furga et al. 1988; Wall et al. 1988). A third interesting case is the pituitary-specific transcription factor GHF-1 (also referred to as Pit-1), which regulates a set of genes, including the growth hormone gene, that apparently are responsible for the cellular phenotype of the anterior pituitary (Bodner et al. 1988; Ingraham et al. 1988; McCormick et al. 1988). Here, we have characterized a tissue-specific transcription factor of the sea urchin that may also be a master regulator of a family of genes that are all expressed in the same cell-type-specific manner as the late H2A-2 and H2B-2 genes during sea urchin ontogeny. Cloning of the TSAP gene may allow us to test this hypothesis.

Materials and methods

Plasmid DNA

The late H2A-2.1 and H2B-2.1 genes were subcloned from cosmid PmL1, and the late H2A-2.1 and H2B-2.2 genes from cosmid PmL2 (Kemler and Busslinger 1986) into the plasmid pSP64. The subcloning strategy and the *in vitro* mutagenesis of the late H2B-2.1 promoter are described in the legends to Figures 5 and 6.

Preparation of nuclear extracts from sea urchin embryos and testes

Nuclear extracts of *Paracentrotus* embryos and testes were prepared as described by Barberis et al. (1987).

Preparation of nuclear extracts from sea urchin tube feet

Tube feet of the sea urchin *P. miliaris* or of the closely related species *P. lividus* were washed in Ca/Mg-free seawater, collected on a nylon net, and frozen directly in liquid nitrogen. All subsequent manipulations were performed at 4°C. Five milliliters of frozen tube feet were blended in a volume of 200 ml of liquid nitrogen in a stainless steel MSE blender for 15 min at high speed, according to a procedure described by Sorger and Pelham (1987). Liquid nitrogen was added every 3 min to main-

tain a volume of 200 ml. The ground tube feet were resuspended in 150 ml of homogenization buffer H [10 mM HEPES (pH 7.9), 15 mM KCl, 1 mM EDTA, 1 mM EGTA, 10% glycerol, 2 M sucrose, 0.15 mM spermine, 0.5 mM spermidine, 1 mM DTT, 0.5 mM PMSF] and homogenized in a Braun MX32 blender for 60 sec, according to the method of Lichtsteiner et al. (1987). The homogenate was passed through a 50- μ m nylon mesh by vacuum filtration. The filtered homogenate was layered in 23-ml aliquots over 10-ml cushions of the same buffer H and centrifuged at 24,000 rpm for 45 min at 0°C in a SW-27 rotor. The pelleted nuclei were resuspended with a Dounce homogenizer (pestle S) in 10 ml of lysis buffer [10 mM HEPES (pH 7.9), 100 mM KCl, 3 mM MgCl₂, 10% glycerol, 1 mM DTT, 0.1 mM EDTA, 0.1 mM PMSF], extracted with 0.36 M (NH₄)₂SO₄ for 30 min, as described by Parker and Topol (1984), and centrifuged at 35,000 rpm for 45 min at 2°C in a Ti60 rotor. The proteins in the supernatant were precipitated by the addition of 0.25 gram (NH₄)₂SO₄ per milliliter of solution and collected by centrifugation at 15,000 rpm for 30 min at 2°C in a Sorvall SS-34 rotor. The protein pellet was dissolved gently in 2 ml of dialysis buffer [20 mM HEPES (pH 7.9), 100 mM KCl, 2 mM MgCl₂, 0.2 mM EDTA, 20% glycerol, 1 mM DTT, 0.1 mM PMSF] per milliliter of extracted nuclei and dialyzed against 500 volumes of this buffer for 5 hr. Insoluble material was removed by centrifugation in a microcentrifuge for 5 min. The extract was stored in aliquots in liquid nitrogen. The protein concentration of these extracts was 0.6–0.8 μ g/ μ l, as determined by the method of Bradford (1976).

Preparation of nuclear extracts from coelomocytes and ovaries

Coelomocytes of the sea urchin *P. lividus* were collected by centrifugation of coelomic fluid at 3500 rpm for 5 min and washed in Ca/Mg-free seawater. Ovaries of the same species were first swirled in 0.55 M KCl to remove as many eggs as possible before washing them in Ca/Mg-free seawater. Both the coelomocytes and the ovaries were resuspended in 150 ml of buffer H and homogenized in a Braun MX32 blender. The nuclei were purified and extracted as described above for the tube foot extract.

In vitro transcription

Transcription reaction (20 μ l) contained 120 ng of circular DNA template (60 ng of the test gene and 60 ng of the reference gene), 1 μ g of plasmid pSP64 DNA, and 50–80 μ g nuclear extract protein in a buffer containing 10 mM HEPES (pH 7.9), 60 mM KCl, 6 mM MgCl₂, 1 mM of each ribonucleotide triphosphate, 10% glycerol, 0.1 mM EDTA, and 0.5 mM DTT. After 50 min of incubation at 25°C, 3 μ l of a mixture containing RNasin (5 units) and DNase I (2 μ g) was added to the transcription reaction, which was then incubated for 15 min at 25°C. DNase I digestion was stopped by the addition of 25 μ l 2 $\times$ proteinase K buffer [200 mM Tris (pH 7.5), 300 mM NaCl, 20 mM EDTA, 2% SDS] and 1 μ l proteinase K (20 mg/ml), followed by a 15-min incubation at 37°C. End-labeled S1 DNA probes were added to this mixture, which was extracted with phenol and chloroform prior to ethanol precipitation. S1 nuclease analysis was carried out as described previously (Busslinger et al. 1981).

DNase I footprint, electrophoretic mobility shift, and methylation interference analyses

DNase I footprint reactions were performed with 1–3 fmoles of end-labeled DNA, as described previously (Barberis et al. 1987). For competition experiments, 2 pmoles of a specific competitor

DNA was included in the reaction mixture prior to the addition of the extract. For electrophoretic mobility shift experiments, 1–3 fmoles of end-labeled DNA was incubated with 3–5 μ g of nuclear protein and 2 μ g of poly[d(I-C)] in 20 μ l of a buffer containing 10 mM HEPES (pH 7.9), 100 mM KCl, 4% Ficoll, 1 mM EDTA, and 1 mM DTT. After incubation for 15 min at room temperature, protein–DNA complexes were separated on a 4% polyacrylamide gel in 0.25 $\times$ TBE buffer at 10 V/cm for 2 hr at room temperature. Competitor DNA was included in the reaction mixture in the amounts described in the legends to Figures 3 and 7.

Methylation interference analyses were carried out in combination with mobility shift experiments using end-labeled DNA fragments that were methylated partially by dimethylsulfate (Maxam and Gilbert 1980). The DNAs in the protein–DNA complex and unbound DNA were eluted from acrylamide, purified on DEAE–cellulose, and cleaved at methylated G and A residues by a G > A reaction. For this, the DNA was dissolved in 20 μ l of 10 mM NaPO₄ (pH 7.0), 1 mM EDTA, heated at 90°C for 15 min, and chilled on ice. Then, 100 μ l of 100 mM NaOH, 1 mM EDTA were added, and the mixture was incubated at 90°C for 30 min. The DNA was neutralized by the addition of 25 μ l of 1 M Tris (pH 7.5), 25 μ l of 2 M NaAc (pH 7) and 100 μ l of 200 mM NaAc (pH 4.8) containing some phenol red prior to ethanol precipitation. The samples were denatured and analyzed on 8% polyacrylamide sequencing gels.

Oligonucleotide probes and competitors

The following oligonucleotides containing the TSAP-binding sites of the late H2A-2 and H2B-2 genes were synthesized:

H2B-2.1: GGAGCACTGATGCAGCGCG
 H2B-2.2: ACCAATACGCAACGAAGCGAAGACGGACCA
 H2A-2.1: GACACGCTTGAAGAAATAGTT
 H2A-2.2: GTGACGCAGCGGTGGGTGACG

The complementary strands of the H2B-2.1, H2A-2.1, and H2A-2.2 sequences were synthesized with a *Bam*HI overhang at their 5' end, and annealed oligonucleotides were cloned into a *Hind*III–*Bam*HI-digested pSP64 vector. The H2B-2.2 oligonucleotide was cloned into the *Hind*III site of the same vector. For DNA probe preparation, these cloned oligonucleotides were excised from the polylinker by *Ava*I and *Pst*I digestion and end-labeled with [α -³²P]dCTP and Klenow DNA polymerase. Twelve copies of the oligonucleotide ACAATACGCAACGAAGCGAAGACGG were all cloned in the same orientation into the *Hind*III site of the pSP64 vector to generate the TSAP-specific competitor DNA containing the binding site of the late H2B-2.2 gene. The OBF-specific competitor was obtained by multimerization of the double-stranded oligonucleotide CTCTCATTTGCATACCAG by T4 DNA ligase.

Injection of sea urchin eggs

Sea urchins of the species *P. lividus* were obtained from the Zoological Station in Villefranche-sur-Mer (France). Eggs were prepared and injected with late histone genes as described previously (Vitelli et al. 1988). Injected eggs were fertilized and cultured for 16–18 hr at 16°C up to the swimming blastula stage. RNA was isolated from these embryos by repeated phenol extraction and ethanol precipitation. Transcripts of the injected late histone genes were analyzed by SP6 mapping as described by Vitelli et al. (1988). The late H2B-2.1 SP6 probe was constructed by subcloning a *Hind*III–*Bam*HI restriction

fragment from position +228 to –56 in the opposite orientation to the direction of SP6 transcription into the polylinker of plasmid pSP64. The late H2A-2.1 SP6 probe was obtained by cloning a *Xho*I–*Pst*I DNA fragment from position +204 to –200 into the polylinker of pSP65.

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References

- Angerer, L., D. DeLeon, K. Cox, R. Maxson, L. Kedes, J. Kaufmeyer, E. Weinberg, and R. Angerer. 1985. Simultaneous expression of early and late histone messenger RNAs in individual cells during development of the sea urchin embryo. *Dev. Biol.* **112**: 157–166.
- Barberis, A., G. Superti-Furga, and M. Busslinger. 1987. Mutually exclusive interaction of the CCAAT-binding factor and of a displacement protein with overlapping sequences of a histone gene promoter. *Cell* **50**: 347–359.
- Birnstiel, M.L., M. Busslinger, and K. Strub. 1985. Transcription termination and 3' processing: The end is in site. *Cell* **41**: 349–359.
- Bodner, M., J.-L. Castrillo, L.E. Theill, T. Deerinck, M. Ellisman, and M. Karin. 1988. The pituitary-specific transcription factor GHF-1 is a homeobox-containing protein. *Cell* **55**: 505–518.
- Bradford, M.M. 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal. Biochem.* **72**: 248–254.
- Busslinger, M. and A. Barberis. 1985. Synthesis of sperm and late histone cDNAs of the sea urchin with a primer complementary to the conserved 3' terminal palindrome; evidence for tissue-specific and more general histone gene variants. *Proc. Natl. Acad. Sci.* **82**: 5676–5680.
- Busslinger, M., N. Moschonas, and R.A. Flavell. 1981. β^+ Thalassemia: Aberrant splicing results from a single point mutation in an intron. *Cell* **27**: 289–298.
- Busslinger, M., D. Schümperli, and M.L. Birnstiel. 1985. Regulation of histone gene expression. *Cold Spring Harbor Symp. Quant. Biol.* **50**: 665–670.
- Childs, G., C. Nocente-McGrath, T. Lieber, C. Holt, and J.A. Knowles. 1982. Sea urchin (*Lytechinus pictus*) late-stage H3 and H4 genes: Characterisation and mapping of a clustered but nontandemly linked multigene family. *Cell* **31**: 383–393.
- Cosson, J., I. Kemler, S. Tannò, and M. Busslinger. 1988. Promoter of a somatic H2B gene of the sea urchin. *Nucleic Acids Res.* **16**: 4175.
- Darnell, J.E., Jr. 1982. Variety in the level of gene control in eukaryotic cells. *Nature* **297**: 365–271.
- Davidson, I., J.H. Xiao, R. Rosales, A. Staub, and P. Chambon. 1988. The HeLa cell protein TEF-1 binds specifically and cooperatively to two SV40 enhancer motifs of unrelated sequence. *Cell* **54**: 931–942.

- Evans, T., M. Reitman, and G. Felsenfeld. 1988. An erythrocyte-specific DNA binding factor recognizes a regulatory sequence common to all chicken globin genes. *Proc. Natl. Acad. Sci.* **85**: 5976–5980.
- Fletcher, C., N. Heintz, and R.G. Roeder. 1987. Purification and characterization of OTF-1, a transcription factor regulating cell cycle expression of a human histone H2b gene. *Cell* **51**: 773–781.
- Flytzanis, C.M., A.P. McMohan, B.R. Hough-Evans, K.S. Kattula, R.J. Britten, and E.H. Davidson. 1985. Persistence and integration of cloned DNA in postembryonic sea urchins. *Dev. Biol.* **108**: 431–442.
- Fried, M. and D.M. Crothers. 1981. Equilibria and kinetics of lac repressor-operator interactions by polyacrylamide gel electrophoresis. *Nucleic Acids Res.* **9**: 6505–6525.
- Galas, D. and A. Schmitz. 1978. DNase footprinting: A simple method for the detection of protein-DNA binding specificity. *Nucleic Acids Res.* **5**: 3157–3170.
- Galson, D.L. and D.E. Housman. 1988. Detection of two tissue-specific DNA-binding proteins with affinity for sites in the mouse β -globin intervening sequence 2. *Mol. Cell. Biol.* **8**: 381–392.
- Garner, M.M. and A. Rezin. 1981. A gel electrophoresis method for quantifying the binding of proteins to specific DNA regions: Application to components of the *Escherichia coli* lactose operon regulatory system. *Nucleic Acids Res.* **9**: 3047–3060.
- Gerster, T., P. Matthias, M. Thali, J. Jiricny, and W. Schaffner. 1987. Cell type-specific elements of the immunoglobulin heavy chain gene enhancer. *EMBO J.* **6**: 1323–1330.
- Halsell, S.R., M. Ito, and R. Maxson. 1987. Differential expression of early and late embryonic histone genes in adult tissues of the sea urchin *Strongylocentrotus purpuratus*. *Dev. Biol.* **119**: 268–274.
- Harvey, R.P., A.J. Robins, and J.R.E. Wells. 1982. Independently evolving chicken histone H2B genes: Identification of a ubiquitous H2B-specific 5' element. *Nucleic Acids Res.* **10**: 7851–7863.
- Ingraham, H.A., R. Chen, H.J. Mangalam, H.P. Elsholtz, S.E. Flynn, C.R. Lin, D.M. Simmons, L. Swanson, and M.G. Rosenfeld. 1988. A tissue-specific transcription factor containing a homeodomain specifies a pituitary phenotype. *Cell* **55**: 519–529.
- Ito, M., J. Bell, G. Lyons, and R. Maxson. 1988. Synthesis and turnover of late H2B histone mRNA in developing embryos of the sea urchin, *Strongylocentrotus purpuratus*. *Dev. Biol.* **129**: 147–158.
- Kaumeier, J.F. and E.S. Weinberg. 1986. Sequence, organization and expression of late embryonic H3 and H4 histone genes from the sea urchin *Strongylocentrotus purpuratus*. *Nucleic Acids Res.* **14**: 4557–4576.
- Kemler, I. and M. Busslinger. 1986. Characterization of two nonallelic pairs of late histone H2A and H2B genes of the sea urchin: Differential regulation in the embryo and tissue-specific expression in the adult. *Mol. Cell. Biol.* **6**: 3746–3754.
- Knowles, J.A. and G.J. Childs. 1984. Temporal expression of late histone messenger RNA in the sea urchin *Lytechinus pictus*. *Proc. Natl. Acad. Sci.* **81**: 2411–2415.
- Knowles, J.A., Z.-C. Lai, and G.J. Childs. 1987. Isolation, characterization, and expression of the gene encoding the late histone subtype H1- γ of the sea urchin *Strongylocentrotus purpuratus*. *Mol. Cell. Biol.* **7**: 478–485.
- LaBella, F., H.L. Sive, R.G. Roeder, and N. Heintz. 1988. Cell-cycle regulation of a human histone H2b gene is mediated by the H2b subtype-specific consensus sequence. *Genes Dev.* **2**: 32–39.
- Lai, Z.-C. and G. Childs. 1988. Characterization of the structure and transcriptional patterns of the gene encoding the late histone subtype H1- β of the sea urchin *Strongylocentrotus purpuratus*. *Mol. Cell. Biol.* **8**: 1842–1844.
- Landschulz, W.H., P.F. Johnson, E.Y. Adashi, B.J. Graves, and S.L. McKnight. 1988. Isolation of a recombinant copy of the gene encoding C/EBP. *Genes Dev.* **2**: 786–800.
- Lichtsteiner, S., J. Wuarin, and U. Schibler. 1987. The interplay of DNA-binding proteins on the promoter of the mouse albumin gene. *Cell* **51**: 963–973.
- Lieber, T., K. Weisser, and G. Childs. 1986. Analysis of histone gene expression in adult tissues of the sea urchins *Strongylocentrotus purpuratus* and *Lytechinus pictus*: Tissue-specific expression of sperm histone genes. *Mol. Cell. Biol.* **6**: 2602–2612.
- Maxam, A.M. and W. Gilbert. 1980. Sequencing end-labeled DNA with base-specific chemical cleavages. *Methods Enzymol.* **65**: 499–560.
- Maxson, R., R. Cohn, L. Kedes, and T. Mohun. 1983a. Expression and organisation of histone genes. *Annu. Rev. Genet.* **17**: 239–277.
- Maxson, R., T. Mohun, G. Gormezano, G. Childs, and L. Kedes. 1983b. Distinct organisations and patterns of expression of early and late histone gene sets in the sea urchin. *Nature* **301**: 120–125.
- Maxson, R., T. Mohun, G. Gormezano, and L. Kedes. 1987. Evolution of late H2A, H2B and H4 histone genes of the sea urchin *Strongylocentrotus purpuratus*. *Nucleic Acids Res.* **15**: 10569–10582.
- McCormick, A., D. Wu, J.-L. Castrillo, S. Dana, J. Strobl, E.B. Thompson, and M. Karin. 1988. Extinction of growth hormone expression in somatic cell hybrids involves repression of the specific trans-activator GHF-1. *Cell* **55**: 379–389.
- Mohun, T., R. Maxson, G. Gormezano, and L. Kedes. 1985. Differential regulation of individual late histone genes during development of the sea urchin (*Strongylocentrotus purpuratus*). *Dev. Biol.* **108**: 491–502.
- Müller, M.M., S. Ruppert, W. Schaffner, and P. Matthias. 1988. A cloned octamer transcription factor stimulates transcription from lymphoid-specific promoters in non-B cells. *Nature* **336**: 544–551.
- Otwinowski, Z., R.W. Schevitz, R.-G. Zhang, C.L. Lawson, A. Joachimiak, R.Q. Marmorstein, B.F. Luisi, and P.B. Sigler. 1988. Crystal structure of *trp* repressor/operator complex at atomic resolution. *Nature* **335**: 321–329.
- Parker, C.S. and J. Topol. 1984. A *Drosophila* RNA polymerase II transcription factor contains a promoter-region-specific DNA-binding activity. *Cell* **36**: 357–369.
- Perry, M., G.H. Thomsen, and R.G. Roeder. 1985. Genomic organization and nucleotide sequence of two distinct histone gene clusters from *Xenopus laevis*: Identification of novel conserved upstream sequence elements. *J. Mol. Biol.* **185**: 479–499.
- Pfeifer, K., T. Prezant, and L. Guarente. 1987. Yeast HAP1 activator binds to two upstream activation sites of different sequence. *Cell* **49**: 19–27.
- Reitman, M. and G. Felsenfeld. 1988. Mutational analysis of the chicken β -globin enhancer reveals two positive-acting domains. *Proc. Natl. Acad. Sci.* **85**: 6267–6271.
- Scheidereit, C., A. Heguy, and R.G. Roeder. 1987. Identification and purification of a human lymphoid-specific octamer-binding protein (OTF-2) that activates transcription of an immunoglobulin promoter *in vitro*. *Cell* **51**: 783–793.
- Siebenlist, U., R. Simpson, and W. Gilbert. 1980. *E. coli* RNA polymerase interacts homologically with two different promoters. *Cell* **20**: 269–281.
- Sive, H.L., N. Heintz, and R.G. Roeder. 1986. Multiple se-

- quence elements are required for maximal *in vitro* transcription of a human histone H2B gene. *Mol. Cell. Biol.* **6**: 3329–3340.
- Sorger, P.K. and H.R.B. Pelham. 1987. Purification and characterization of a heat shock element binding protein from yeast. *EMBO J.* **6**: 3035–3041.
- Staudt, L.M., R.G. Clerc, H. Singh, J.H. LeBowitz, P.A. Sharp, and D. Baltimore. 1988. Cloning of a lymphoid-specific cDNA encoding a protein binding the regulatory octamer DNA motif. *Science* **241**: 577–579.
- Staudt, L.M., H.S. Singh, R. Sen, T. Wirth, P.A. Sharp, and D. Baltimore. 1986. A lymphoid-specific protein binding to the octamer motif of immunoglobulin genes. *Nature* **323**: 640–643.
- Superti-Furga, G., A. Barberis, G. Schaffner, and M. Busslinger. 1988. The –117 mutation in Greek HPFH affects the binding of three nuclear factors to the CCAAT region of the γ -globin gene. *EMBO J.* **7**: 3099–3107.
- Vitelli, L., I. Kemler, B. Lauber, M.L. Birnstiel, and M. Busslinger. 1988. Developmental regulation of micro-injected histone genes in sea urchin embryos. *Dev. Biol.* **127**: 54–63.
- Wall, L., E. deBoer, and F. Grosveld. 1988. The human β -globin gene 3' enhancer contains multiple binding sites for an erythroid-specific protein. *Genes Dev.* **2**: 1089–1100.
- Wirth, T., L. Staudt, and D. Baltimore. 1987. An octamer oligonucleotide upstream of a TATA motif is sufficient for lymphoid-specific promoter activity. *Nature* **329**: 174–177.
- Wu, H.-M. and D.M. Crothers. 1984. The locus of sequence-directed and protein-induced DNA bending. *Nature* **308**: 509–513.
- Zweidler, A. 1984. Core histone variants of the mouse: Primary sequence and differential expression. In *Histone genes and histone gene expression*. (ed. G. Stein, J. Stein, and W. Marzluff), pp. 339–371. John Wiley, New York.

Developmental and tissue-specific regulation of a novel transcription factor of the sea urchin.

A Barberis, G Superti-Furga, L Vitelli, et al.

Genes Dev. 1989, **3**:

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References

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