Sequences closely related to an immunoglobulin gene promoter/enhancer element occur also upstream of other eukaryotic and of prokaryotic genes

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Received 1 September 1986; Revised and Accepted 17 October 1986

ABSTRACT
Decanucleotide sequences closely related to the TNATTTGCAT element which occurs upstream of the immunoglobulin genes and in the immunoglobulin gene enhancer were found also upstream of other eukaryotic and of prokaryotic genes. The possibility of evolutionary and functional relationships between the various transcriptional systems is discussed.

INTRODUCTION
The highly conserved sequence TNATTTGCAT (dc; ref. 2) occurs upstream of the immunoglobulin light chain genes and, in the inverse orientation (ATGCAAATNA; cd), in the corresponding region of the heavy chain genes (3, 4). These dc and cd sequences have been shown to be necessary promoter elements for the transcription of immunoglobulin genes (4-7) and they appear to bind specifically (a) nuclear protein(s) (8, 9). Also the dc sequence in the immunoglobulin heavy chain enhancer (4) was found, by in vivo (10) and in vitro assays (8), to be a site of specific protein binding.

We previously noticed (4, 11-13) that dc and cd related sequences occur also in eukaryotic gene regions unrelated to the immunoglobulin genes and in viral enhancers (e.g. 14), upstream of histone genes (15) and of various Drosophila genes (13, 16, 17). Recently cd related elements upstream of the U1 and U2 small nuclear RNA genes were found to possess enhancer like activity (18, 19). We now report that a number of prokaryotic genes, some of them responsive to cAMP, carry dc or cd related sequences within their regulatory regions.
RESULTS AND DISCUSSION

The conserved regions upstream of the immunoglobulin genes have been defined as octanucleotides by Parslow et al. (3) and as decanucleotides by us (4). Our definition rests on comparisons of 12 (ref.11) or 22 light chain gene regions. The latter comparison (12) yielded the following consensus sequence: T_{18}^NA_{19}(TTTGC)_{22}A_{20}T_{22}. On this basis it is possible to call the element a hepta, octa, nona, or decanucleotide. Since only beyond the decanucleotide the homology drops to low values we prefer the latter designation. It should be noted that also in most immunoglobulin heavy chain and histone gene regions 10 bp are conserved.

In addition to the dc element we observed, further upstream of the light chain genes, a sequence of 15 nucleotides which is less well conserved than the dc sequence (4). The extent of conservation is clearly significant, however, in comparisons of 12 (ref.11) or 22 light chain genes (ref.12; T_{19}G_{20}C_{21}A_{18}C_{16}T_{19}G_{18}T_{14}G_{14}NC_{13}C_{15}A_{17}G_{15}). The 15 bp sequence can be removed without loss of transcriptional activity in transfection assays (5). It cannot be excluded, however, that it plays a role in some as yet undetected regulatory processes.

Homologies between prokaryotic and eukaryotic promoter elements

In Table 1 the CRP binding regions of prokaryotic genes are compared with the dc regions found in eukaryotic promoter/enhancer regions. No systematic search of other known E. coli DNA sequences for the occurrence of dc-like sequences has been carried out.

Sequence homologies of 80-100% are found between several eukaryotic dc regions and parts of the CRP binding region of the E. coli crp gene. This gene codes for the cAMP receptor protein and is subject to negative autoregulation (20). The homologies extend beyond the decanucleotide sequences. Most remarkable are homologies between 19 bp segments of the VK MPC 11, the Xenopus U1B, and the prokaryotic regulatory region (only 2 and 3 mismatches respectively; Table 1, upper part).

The decanucleotide located in the CRP binding region of the ara BAD/ara C operon shows homologies of 70-90% to several eukaryotic dc elements (Table 1, middle part). The ara BAD operon which codes for enzymes of the arabinose metabolism and the ara C
Homologies between prokaryotic and eukaryotic promoter elements. The CRP binding sites (indicated by large brackets) of the E. coli crp gene (Eco crp), the ara BAD operon (Eco ara) and a possible CRP binding site of the E. coli car AB operon (Eco car) are significantly homologous to promoter elements of murine (m), human (h), chicken (chk), sea urchin (su), Xenopus (xen) and viral (SV40) genes. The homologues (hom) which include the dc regions (boxed) are located between the gaps; the homologues are expressed in matched versus mismatched base pairs. The numbers in the ATG column refer to the distance between the first nucleotides of the dc or cd region and the start codon. The TGTGA motif (or the complementary form TCACA) which is found in most CRP binding sites (21) is underlined; related sequences are indicated by broken lines; note that the motif occurs in some gene regions upstream and in others downstream of the dc sequence. An additional 7 bp homology between Eco crp and xen U1B is indicated by dots. In the VK HPC II region a dash was introduced to maximize homology; this deletion was scored as a mismatch. The non-transcribed DNA strand is shown in the usual 5'-3' orientation (start codon on the right side); an * indicates that, for a cd element, the transcribed strand is shown in the inverse direction (start codon on the left side). The references are listed on the right side.
Homologies between enhancers and upstream elements of prokaryotic and eukaryotic genes. The simian virus 40 enhancer (SV40 EH), the mouse immunoglobulin heavy chain enhancer (m IgH EH) and the Xenopus U2 gene enhancer (xen U2 EH) are compared to murine (m), human (h), chicken (chk), calman (cal), Xenopus (xen), and E. coli (Eco) genes. The decanucleotide sequence is boxed. The abbreviations used and the arrangement of the Table are the same as those of Table 1.

In Table 2 the dc regions of viral and cellular enhancers are compared to the sequence elements found in the 5' flanking regions of prokaryotic and eukaryotic genes. Three sequence pairs which are listed in Table 1 are shown again in order to allow a direct comparison between the prokaryotic and the additional eukaryotic sequences.

The dc element of the SV40 enhancer is located about 30 bp downstream of the enhancer core (14). It is part of an alternating Pu/Py stretch. This region shows excellent homology to promoters of the immunoglobulin genes, the chicken and sea urchin histone H2B genes, and the E. coli crp gene (Table 2, ref. 35).
In the SV40 enhancer the dc sequence seems to be functionally important \(^{51,52}\). It may respond to the same cellular signals as the eukaryotic upstream elements.

The dc region of the mouse IgH enhancer is related more closely to the immunoglobulin gene promoters than to the other eukaryotic dc regions (Table 2, middle part). It has been suggested to be a protein binding site because in studies carried out on lymphoid cells in vivo the G residue in the enhancer dc element is protected against methylation \(^{10}\). A recent report provides evidence that the dc region of the IgH enhancer serves as a promoter element of 'sterile' c\(\mu\) transcripts which contain constant region sequences but lack variable gene sequences \(^{23}\).

The Xenopus U2 gene enhancer element is fairly homologous to the dc elements of VH genes of caiman (Table 2, lower part). This may reflect the close relationship of this phylogenetically ancient reptile \(^{24}\) to amphibians. The U2 gene enhancer is interesting in another respect: when a synthetic 14 bp oligonucleotide comprising the dc region is cloned upstream of the U2 gene it stimulates transcription (independent of orientation) in the Xenopus oocyte transcription system \(^{18}\), see also refs. 19, 25,26). This parallels the situation found for the dc elements in immunoglobulin gene promoters: they are necessary for correct and efficient transcription.

It should be noted that not only the dc sequence but also the GGGCGG element occurs both in promoters and enhancers; also promoter sequences were found to act as enhancers when being transposed to other parts of the gene regions (review ref. 53). Recent experiments suggest a unified view of gene regulation by proteins that bind to sites on the DNA either nearby or at a considerable distance (review 54).

Are elements of transcriptional control mechanisms conserved between prokaryotes and eukaryotes?

Although the common sequence elements are short, the statistical probability of their chance occurrence at an analogous position in the regulatory regions of unrelated genes is very low. An approximate calculation (for details see refs. 27,28) shows that the probability of chance occurrence (termed C) of the 19/2 (19/3) homology anywhere in the same 80 bp region (250 bp region)
of the crp gene and the VK MPC 11 (Xenopus U1B) gene is less than $10^{-6}$ ($10^{-4}$). The probability of finding such a homology in the promoter region of a third unrelated gene is even less likely ($C^2$). The location of dc/cd related sequences within protein binding regions, both in eukaryotes and prokaryotes, and the unlikelihood of a chance occurrence lead to the conclusion that the sequences are evolutionarily related.

Since the dc or cd containing prokaryotic promoter regions belong to cAMP responsive genes one may ask whether the occurrence of dc/cd in eukaryotic promoter regions has also a functional meaning in the sense of cAMP responsiveness. In this context the recent study of Nagamine and Reich (29) should be mentioned: homologies between the regulatory regions of prokaryotic cAMP responsive genes (including ara BAD) and putative regulatory regions of eukaryotic cAMP responsive genes were detected. The regions of homology, however, do not include dc or cd related sequences (although in ara BAD a cd related element is located adjacent to the observed homology region). A dc related sequence occurs in the beginning of the transcribed region of rat tyrosine amino transferase (30) and a cd related sequence is found upstream of a rat phosphoenolpyruvate carboxykinase gene (31). There are, however, prokaryotic and eukaryotic cAMP responsive genes in which no dc/cd related sequences have been detected. On the other hand, such obviously unrelated eukaryotic genes with dc/cd containing promoters as the immunoglobulin and the histone genes are not known to be cAMP responsive. Still it is likely that the occurrence of dc and cd related elements in the promoter or putative promoter regions of both, eukaryotic and prokaryotic genes has a functional importance perhaps mediated by a particular DNA conformation. One may postulate that the dc and cd related sequences are conserved between prokaryotic and eukaryotic gene regions as sites of interaction with regulatory proteins; at least the domains of the regulatory proteins which interact with the sequences should also be conserved. Although at present there exists no experimental evidence, not even an indication, that these domains belong to cAMP responsive proteins, one may keep also this possibility in mind.
Cell type specificity of the immunoglobulin gene promoter

The dc/cd related sequences upstream of the immunoglobulin gene have not only been found to possess a promoter function but they have also been postulated to contribute to the fact that these genes are expressed practically only in lymphoid cells (6,7,11,32,33). However, for two reasons the dc/cd related sequences cannot be the only determinant of the tissue specificity of immunoglobulin gene expression: the sequences occur also in the promoter regions of e.g. histone and small nuclear RNA genes which are expressed in many different cell types; also non-lymphoid cells like HeLa cells contain (a) protein(s) which bind specifically to dc/cd related sequences and are thought to possess a regulatory function (8,9). Apparently the dc/cd elements are necessary but not sufficient for the cell type specificity of immunoglobulin gene expression. Other upstream elements as the above mentioned 15 nucleotide long sequence (4) or sequences within the immunoglobulin genes themselves may play a role. Additional B cell specific factor(s) may bind to these sequences or act at the chromatin level. Also the dc/cd binding protein(s) (8,9) may be controlled in analogy to the E. coli CRP, by (tissue specifically produced) secondary signals.

It is altogether likely that most proteins have evolved from a very small number of archetypal proteins (34). This may also be true for the evolution of transcriptional systems. Therefore common ancestry should be the cause of the similarities between the prokaryotic and the eukaryotic transcriptional systems, although a convergent evolution to homologous structures cannot be ruled out.

ACKNOWLEDGMENT

The work was supported by Bundesministerium für Forschung und Technologie and Fonds der Chemischen Industrie.

REFERENCES

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2. Abbreviations: VK and VH, variable regions of kappa light chain and heavy chain genes; dc and cd, decanucleotide elements (see text); CRP, cAMP receptor protein of E. coli. Other abbreviations are listed in the figure legends.