

- a time-frame for achieving the elimination of nuclear weapons. This it did with its Quinquennial Statement of Goals, in 1997, when a period of not more than twenty years was mentioned for the first time. In recent years, it has organized several workshops on the theme of a NFWF, in particular the one in Delhi in March 1998.
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REVIEW ARTICLES

New insights into the multiple functions of Sp1, a ubiquitous transcription factor

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The eukaryotic transcription factor Sp1 is a sequence-specific DNA-binding protein that binds to GC-rich sequences in a large number of promoters. Despite its ubiquitous nature, Sp1 (and related factors) can modulate transcription of specific genes through interactions with cell type or stage-specific transcription factors. Sp1 can also directly influence formation of the transcription initiation complex. Furthermore, binding of Sp1 at distal promoter elements can affect formation of active chromatin structures. Thus the Sp1 family of proteins plays a crucial role in gene regulation in higher organisms.

THE patterns of gene expression in cells of higher organisms require thousands of genes to be turned on or off in specific cell types in a temporally regulated manner. This regulation most often occurs at the level of tran-

scription. The transcription of nuclear genes is catalysed by three distinct RNA polymerases (I, II and III). RNA polymerase II (pol II) is required for the transcription of genes encoding mRNAs and certain small nuclear RNAs. Although pol II is a multisubunit enzyme, it also needs a set of additional proteins known as general transcription factors for promoter recognition. Prominent amongst these factors is the TFIID complex, of which the TATA box binding protein is an essential component. In addition to the core or basal promoter elements such as the TATA box, pol II promoters also contain essential promoter proximal sequences (within 200 bp of the initiation site) and distal enhancer sequences. Specific protein-DNA interactions are required at each of these elements for precise initiation of transcription of a particular gene by pol II. Transcriptional activators bind to promoter proximal elements to modulate transcription

several-fold over the basal level. This effect may be direct or indirect, and ultimately results in enhancement of one or more of the following: rate of formation of initiation complex, its stability or its catalytic functions. The basic mechanism of transcription initiation by pol II has been extensively reviewed earlier¹⁻³.

Ubiquitous transcriptional activators include various classes of transcription factors such as Sp1, AP1, AP2 and E2F. These factors appear to exert promoter-specific regulation through the formation of unique complexes with other factors depending on the arrangement of binding sites within a particular regulatory region. In this article, recent developments in our understanding of the mechanisms of regulation by the Sp1 family of transcription factors are reviewed.

Characteristics of Sp1

Sp1 was one of the first eukaryotic transcription factors to be identified and was the first transcription factor for which the gene was cloned. In pioneering studies by Tjian and coworkers, Sp1 was initially characterized as a host factor from HeLa cells that bound in a specific manner to the SV40 (Simian virus 40) early promoter at GC-rich sites and was essential for *in vitro* transcription of this promoter^{4,5}. Purified Sp1 consists of a single polypeptide chain of 778 amino acids and exhibits an apparent molecular mass of 105 kDa. Sp1 is an abundant nuclear protein found in many cell types in higher eukaryotes.

Sp1 has the potential to activate transcription from a large number of cellular promoters that contain GC-rich sequences. Based on earlier studies⁶, a decanucleotide consensus sequence that is characteristic of Sp1 binding sites has been defined: 5'-G(T)GGGCGGG(A)G(A) C(T)-3'. Sp1 binding sites are functional in either orientation. Recent studies indicate that the Sp1 family of transcription factors can also bind to GT-rich sequences^{7,8}. An unusual binding site for Sp1 bearing the sequence: 5'-TCCTCC-3' has been described for the $\alpha 2(1)$ collagen gene promoter⁹. Many promoters responsive to Sp1 contain a series of Sp1 binding sites, although certain promoters can be activated by a single GC box.

The DNA binding activity of Sp1 has been attributed to a region of the protein containing three Zn fingers^{10,11} with the structure Cys₂-His₂ (Figure 1). Although the crystal structure of Sp1 bound to its DNA motif is not available, Zn fingers occur in a large variety of transcription factors and interact with DNA in a similar fashion. Data from known structures^{12,13} suggest that a run of fingers wraps around the DNA, and the N-terminal portion of the α -helix from each finger projects into the major groove. The DNA-binding region of Sp1 is distinct from the regions of the protein involved in

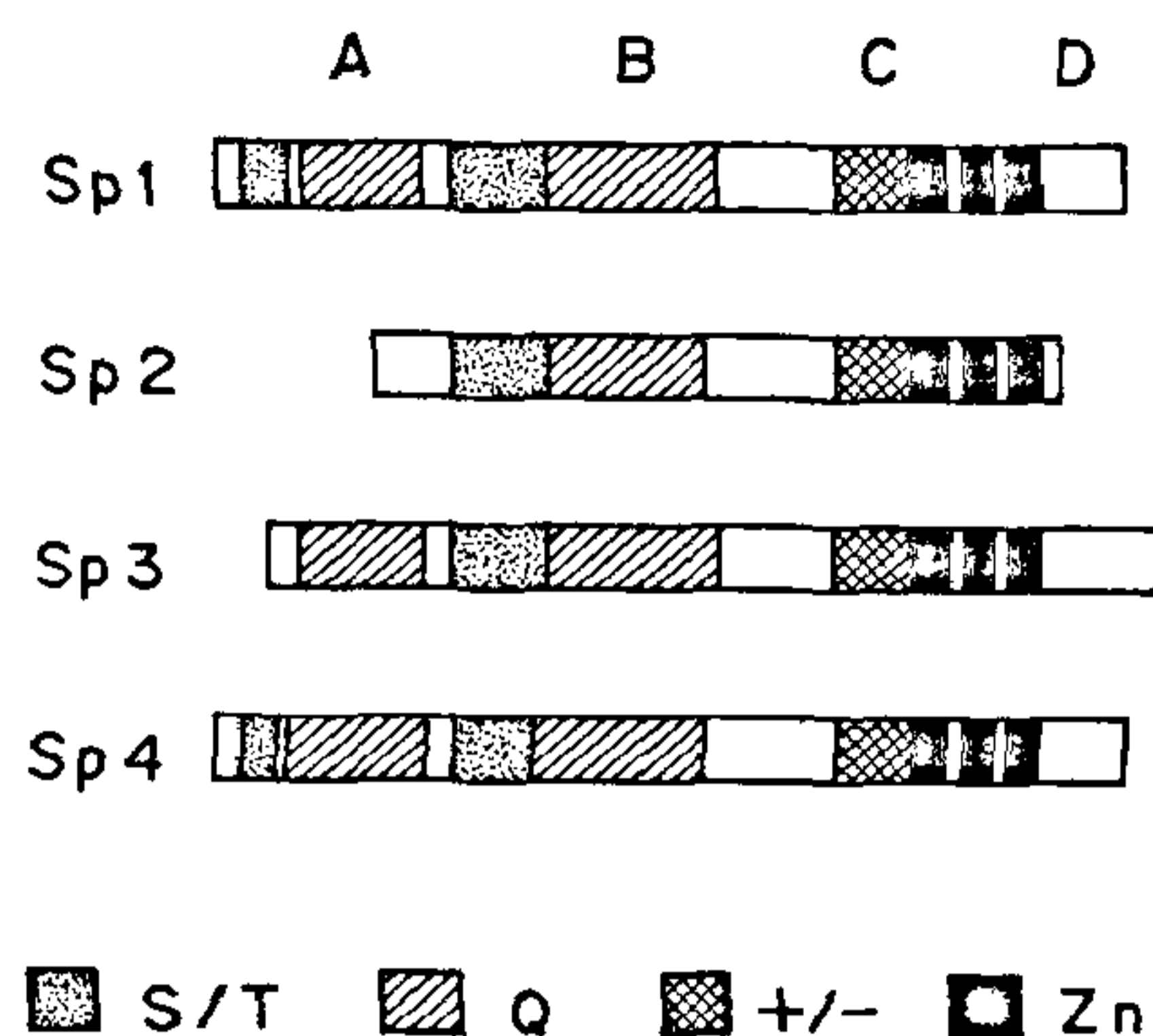


Figure 1. Protein domains of Sp1, Sp2, Sp3 and Sp4. A, B, C, D transactivation domains. S/T, serine/threonine-rich; Q, glutamine rich; +/-, highly charged; Zn, Zn fingers. (Data adapted from refs and 8).

activation of transcription. Four transactivation domains have been identified in Sp1 (ref. 14) and these are indicated in Figure 1. Two of these domains which are towards the N-terminus have subdomains rich in serine/threonine and glutamine, whereas a third domain is highly charged. Evidence for the interaction of different regions of Sp1 protein with various transcription factors is presented in a later section. Sp1 has been shown to be phosphorylated¹⁵ and highly glycosylated¹⁶ but the functional significance of these modifications is not clear.

Sp1-related proteins

The existence of proteins similar to Sp1 has been demonstrated in different studies. Analysis of a T-cell antigen receptor promoter revealed a critical GT-rich element that could bind to Sp1 as well as two other proteins⁸. These new proteins were shown to be coded by two separate genes and have been called Sp2 (~ 80 kDa) and Sp3 (~ 100 kDa). Studies with the uteroglobin promoter GT box⁷ led to the identification of two Sp1-like proteins which were later termed Sp3 and Sp4 (ref. 17). The protein structures of Sp2 and Sp3 are very similar to that of Sp1 (ref. 8) (see Figure 1). All three proteins contain Zn fingers with the structure Cys₂-His₂. At the Zn finger domain, homology with Sp1 is 72% for Sp2 and 90% for Sp3. Sp2 has limited homology to Sp1 outside this domain; however, Sp3 has extensive homology to Sp1 throughout the coding region. The Sp2 protein has a serine/threonine-rich region followed by a glutamine-rich domain (B), a highly charged domain (C) and three Zn fingers. On the other hand, Sp3 and Sp4 are very similar to Sp1, with two homologous glutamine-rich regions, a serine/threonine-rich region, a highly charged domain, three Zn fingers

and a C-terminal domain similar to the Sp1 domain D. Sp2 and Sp3 are widely expressed in different cell types, as is Sp1. However, Sp4 is highly expressed only in the brain, suggesting a tissue-specific role for this factor⁷. Sp3 and Sp4 proteins can bind to both GT-rich and GC-rich DNA motifs with high affinity, whereas Sp2 binds with lower affinity to a GT box motif and not at all to the GC box motif. Sp4 also shows a high degree of structural conservation with Sp1 and Sp3.

The striking similarity between Sp1 and Sp3 raises the issue of their respective roles in the activation of a specific promoter *in vivo*. Studies of promoter activation by Sp1/Sp3 in transient DNA transfection assays in mammalian cells are complicated by the high endogenous levels of these factors in most cell types. Hence such experiments are normally carried out in *Drosophila* cultured cells (Schneider line 2) which are deficient in Sp1 and its homologs^{14,18}. Using this system, it has been possible to identify promoters responsive to either Sp1 or Sp3. The leukocyte integrin gene can be activated by either Sp1 or Sp3 (refs 19, 20). The induction of the cyclin-dependent kinase inhibitor p21 during keratinocyte differentiation requires Sp3 specifically, although both Sp1 and Sp3 contribute to the basal activity of the promoter²¹. Certain promoters have been found to respond to both Sp1 and Sp3 synergistically, such as in the case of the neuronal nicotinic acetylcholine receptor β 4 subunit gene²². There have been several reports of inhibition by Sp3 following the initial finding that Sp3 acts primarily as a repressor of promoter activity²³. However, some of these studies may need to be re-evaluated in light of recent evidence that Sp3 mRNA can code for smaller polypeptides (~ 80 kDa) via translational initiation at two internal start sites located within the Sp3 transactivation domain, which can function as potent inhibitors of Sp1 or full-length Sp3-mediated transcription²⁴. Hence transient transfection assays employing Sp3 expression vectors yielding full length protein would score Sp3 as an activator whereas vectors yielding internally initiated isoforms would score Sp3 as an inhibitor.

Functional roles of Sp1

Activation through independent sites

Sp1 was discovered by identification of its ability to selectively increase transcription *in vitro* from the SV40 early promoter^{4,5}. Six GC boxes are present in tandem at this promoter. By making point mutations in each of these sites and studying their capacity for transcription *in vitro*, it was determined that five of these boxes are essential for transcription and as shown by DNAase I footprinting analysis can bind to Sp1 simultaneously. It was observed that mutations at specific GC boxes did

not affect interactions at adjacent sites²⁵. Although the binding affinity of Sp1 for these different sites varied considerably, there was no evidence for cooperative interactions. There are several examples of promoters that contain one or more independent Sp1 sites. However, there has been considerable interest in recent times in the ability of Sp1 to interact with other factors to modulate transcription in various ways (see Figure 2). Some examples of such cooperative interactions are discussed in the following sections.

Synergistic transactivation through transcription factors

Small changes in the concentrations or availability of transcription factors can lead to profound changes in patterns of gene expression. One of the primary mechanisms that has been proposed to be involved is that of synergistic interactions between factors bound to different motifs within a transcriptional control region, so that the final level of activation is considerably higher than an additive effect. In addition, if one of the factors is specific to a cell type, such an activation can lead to tissue-specific gene expression. There are many examples of synergistic interactions between transcription factors, some involving Sp1 as discussed below. It is generally thought that a higher order complex provides a more effective structure for assembling or triggering initiation by the basal machinery. Current ideas on how this might be brought about are examined at the end of this review.

The presence of four separate activation domains in Sp1 has led to several questions on the roles of each of these domains. Evidence for their role in different kinds of synergistic transactivation is discussed in this section.

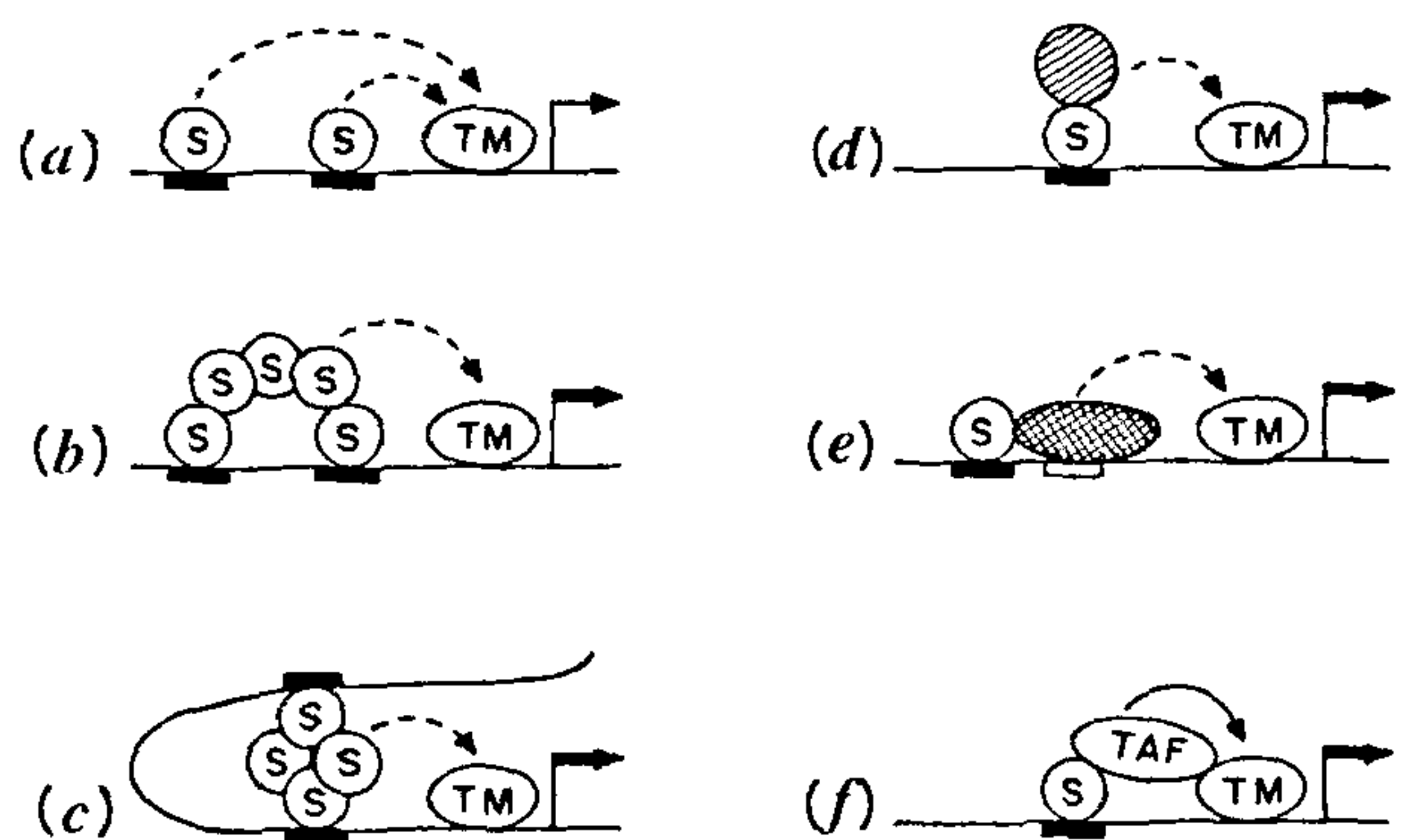


Figure 2. Mechanisms for effects of Sp1 on transcription. *a*, Simple activation by binding of Sp1 at independent sites^{4,5}; *b*, Synergistic activation by multimers of Sp1 (ref. 26); *c*, Synergistic activation by Sp1 bound at distal and proximal sites²⁷; *d*, Superactivation by Sp1 (ref. 28); *e*, Synergism of Sp1 with growth-regulatory or cell-specific transcription factors^{30,31}; *f*, Direct effects on initiation of transcription^{38,39}. S, Sp1; TM, transcription machinery; TAF, TATA-binding protein associated factor.

Multimerization of Sp1: Many transcription factors are known to multimerize to form an active species. Although DNA binding by Sp1 does not require dimerization, it is now evident that two adjacent Sp1 sites can act synergistically to activate transcription nearly 100-fold in synthetic or test promoters²⁶. However, the synergistic activation by Sp1 is clearly dependent on the specific promoter, as there is no synergism in the case of the SV40 early promoter which has six adjacent Sp1 sites that work independently from each other. Studies with Sp1 mutants deleted in one or two transactivation domains indicate that three of the domains (A, B and D) are required for synergistic activation on two adjacent binding sites in a proximal promoter construct. Domains A and B are also required for activation at a single site, unlike domain D. All three domains are necessary for long-range synergistic activation between a proximal site and a distal (enhancer) site.

Superactivation by Sp1. A non-DNA binding mutant of Sp1 has been observed to enhance the ability of a wild-type Sp1 molecule (that binds to DNA) to activate transcription²⁷. This process, called superactivation, has been shown to involve Sp1-Sp1 interactions and requires domains A and B²⁶. In this case, these interactions may be a subset of those necessary for synergistic activation of Sp1 multimers.

An important transcriptional regulator that causes superactivation of Sp1- and Sp3-mediated transcription is the retinoblastoma (Rb) protein^{28,29}. Rb has been shown to regulate transcription of key growth control genes via its physical interaction with a number of sequence-specific transcription factors, but Rb itself does not bind specifically to DNA. Promoters responsive to activation by Rb and Sp1/Sp3 contain the core sequence 5'-GCCACC-3', and include the *c-fos*, *c-myc* and *TGF-β1* gene promoters. The regions of the Rb protein that interact with Sp1/Sp3 to cause superactivation include those amino acids that are mutated in human tumours and are known to be required for Rb-mediated growth suppression of tumour cells in culture. In the absence of functional Rb, Sp1/Sp3 are unable to mediate activation of key growth regulatory genes such as *c-fos*, *c-myc* and the *TGF-β1* gene.

Synergistic interactions with growth-regulatory transcription factors. A second class of growth regulatory transcription factors is the E2F family of proteins. E2F family members interact with Rb when Rb is hypophosphorylated and E2F-mediated transcription is suppressed. When Rb is phosphorylated in mid-G1, E2F is released and can activate transcription. In the case of two DNA replication-associated enzymes, thymidine kinase and dihydrofolate reductase, there is considerable evidence that E2F and Sp1 can physically interact to activate transcription of the corresponding promoters.

Coimmunoprecipitation studies, binding assays and deletion analysis suggest that domain D and the zinc finger region of Sp1 are essential for interaction with E2F at the dihydrofolate reductase gene promoter³⁰ as well as the thymidine kinase gene promoter³¹. This association between Sp1 and E2F is maximal in cells in mid- to late-G1, when E2F is dissociated from Rb and before it associates with cyclin A³⁰. What is the role of Sp1-E2F interactions? An interesting hypothesis is that since E2F is known to bind to TATA-binding protein, E2F may act as a bridging factor transmitting the activation signal from Sp1 to TATA-binding protein in a growth- and cell cycle-dependent manner³¹.

We have been studying the growth-associated activation of the lamin A gene³²⁻³⁴. The rat lamin A promoter contains three Sp1-binding motifs and an AP1-binding site. Both c-Jun and c-Fos which are early response growth-regulatory transcription factors, can bind to this AP1 motif. In dividing cells, binding of Sp1 and AP1 is essential for maximal promoter activity. However, in quiescent cells that do not express lamin A, there is no detectable binding of AP1 to its motif.

Synergistic interactions with cell-specific transcription factors. Interactions between a ubiquitous transcription factor like Sp1 and a cell-specific transcription factor can lead to high levels of cell-specific transcription. This was initially demonstrated with GATA-1, the major erythroid transcription factor³⁵ and has now been demonstrated for other transcription factors as well. GATA-1 and Sp1 binding sites are found in many erythroid cell expressed promoters. The regions mediating physical interactions between GATA-1 and Sp1 have been shown to correspond to specific Zn fingers in their DNA binding domains which are not essential for DNA-binding. In other studies, Sp1 has been shown to interact with cell-specific transcription factors such as HNF3α in lung epithelial cells³⁶ and the myeloid-specific factor PU.1 (ref. 37). These examples document how the combinatorial action of a cell-specific factor and a ubiquitous factor can lead to high levels of cell-specific expression of a promoter.

Direct effects on initiation of transcription

Direct interactions of a transcriptional activator with components of the basal transcription machinery can lead to high levels of activation. These interactions can also be synergistic in nature. The glutamine-rich domains A and B of Sp1 have been shown to interact with a TBP-associated factor, TAF_{II}110, via hydrophobic residues present in these domains, and mediate transcriptional activation³⁸. Interactions have also been shown to occur between the DNA-binding domain of Sp1 and TAF_{II}55 (ref. 39). TAF_{II}55 is an example of a

coactivator that mediates a response to multiple transcription factors, including Sp1, YY1 and others. Thus these interactions can lead to a direct linkage between Sp1 and the initiation complex. This may have important implications for the functioning of TATA-less promoters, which often have several Sp1 binding sites. The hamster dihydrofolate reductase gene promoter does not have a TATA box but has four Sp1 binding sites⁴⁰. Three of these sites control the choice of transcription initiation site and the relative use of the major and minor initiation sites. As discussed earlier, interactions of the proximal-most Sp1 site with E2F control high levels of cell cycle-dependent transactivation of the promoter. Thus different features of the promoter are required for high levels of specific transcription. Similarly, the TATA-less carbamoyl phosphate synthase gene promoter contains two essential Sp1 sites, of which the more proximal motif is required for correct selection of the initiation site⁴¹.

Negative regulation by Sp1

It is becoming increasingly evident that Sp1 can act as a negative regulator of transcription in certain instances. For example, in the proximal promoter of the human adenine nucleotide translocase 2 (*ANT2*) gene, an Sp1 site situated from -7 to -2 bp inhibits transcription, probably by disrupting the recruitment and assembly of the transcriptional initiation complex⁴². In the case of regulation of the megakaryocyte-specific α_{IIb} subunit of the integrin receptor⁴³, a silencer element in the proximal promoter has been shown to bind specifically to Sp1. The authors have presented a model for regulation of this promoter that invokes inhibition of transcription initiation in non-megakaryocytic tissues by Sp1. In megakaryocytes, this silencing effect is overcome by binding of tissue-specific factors such as GATA and Ets proteins. The silencing effects of Sp1 may require a series of complex interactions that are not well understood at present. For instance, the above two promoters also have other Sp1-binding sites that activate transcription, and the relative effects must be context-dependent.

Activation of chromatin

Earlier studies have shown that Sp1 can establish interactions between distal regulatory elements and proximal sites in the SV40 early promoter *in vivo* by looping out the intervening DNA²⁷. In the human β -globin locus control region, Sp1 binding motifs in the crucial 5'HS3 site are likely to be required for remodelling the chromatin structure of this region in erythroid cells⁴⁴. Furthermore, it has been observed that Sp1 can directly or indirectly prevent methylation of CpG islands (CG-rich sequences) in mammalian cells^{45,46}. CpG islands of

housekeeping genes are constitutively unmethylated in all cell types and this is necessary for the activity of these genes. In addition to carrying out *de novo* methylation of DNA, embryonic cells (unlike somatic cells) also protect CpG islands from methylation through specific factors that protect DNA from methylation or actively demethylate it. In the adenine phosphoribosyl transferase gene, Sp1 elements near CpG islands are essential for protecting them from *de novo* methylation. Mutated Sp1 elements introduced into the gene result in its *de novo* methylation in transgenic animal models. Thus Sp1 appears to be one of the factors responsible for establishing the correct genomic methylation pattern required for regulating basal gene expression in the organism.

Analysis of Sp1 function by gene targeting studies

Since Sp1 has been implicated in a wide range of activities in addition to its direct role in transcriptional activation, it is important to obtain evidence for its biological role in the whole animal. Towards this end, the mouse *Sp1* gene has been inactivated by gene targeting via homologous recombination⁴⁷. The *Sp1* knock-out causes lethality around day 9.5 of gestation. *Sp1*-deficiency was found to cause general cellular malfunction resulting in abnormal development with heterogeneous defects. This reflects an indispensable function for Sp1 in every cell type. Thus Sp1 is absolutely essential for normal mouse development. However, it was noted that certain putative target genes of Sp1 were expressed normally, suggesting that other members of the Sp1 family of genes might compensate in part for the loss in Sp1 activity. *Sp1*-negative cells were found in early embryonic stages but not after differentiation had occurred. Thus Sp1 may be essential for regulation of events during differentiation. Two important genes that were found to be expressed at lower levels in the *Sp1*-negative embryos were the thymidine kinase gene and the *MeCP2* gene which codes for a protein that binds to methylated CpGs and might act as a transcriptional repressor. The *MeCP2* knock-out results in a phenotype that is very similar to the *Sp1* gene knock-out⁴⁸. Hence *MeCP2*-deficiency may contribute significantly to the phenotype of the *Sp1* gene knock-out.

Conclusions and future prospects

This review highlights the different functions that can be performed by a single family of transcription factors. Despite its ubiquitous nature, Sp1 can act in a temporal and cell-specific manner through a number of context-dependent interactions. The primary mechanism involved in many instances is synergistic transactivation.

The two main features of synergy are that activation by multiple activators is greater than an additive effect and the response to increasing activator concentrations is sigmoidal. These effects are seen both at proximal promoters and enhancers. How is synergy in transcription brought about? Studies on the T-cell receptor α chain and interferon β gene enhancers have led to the following model⁴⁹⁻⁵². Initially activators bind to DNA in a multistep process that exhibits cooperativity at each step. This is followed by protein-protein interactions between activators to form a stable complex. This complex, called the enhanceosome, presents a distinct surface complementary to that displayed by the pol II basal machinery and coactivators. This leads to cooperative interactions between the complexes and results in synergistic transcription. The enhanceosome model is based on the mode of action of a limited set of enhancers. Further studies need to be carried out to determine whether it is broadly applicable. For instance, it needs to be verified whether the biochemical details of enhanceosome formation are applicable to Sp1-binding at specific promoters. The ability of Sp1 to act as an activator as well as a repressor by binding to different motifs on the same promoter is intriguing and needs to be understood in more detail. Furthermore, we do not fully understand the mechanism of Sp1-mediated initiation of transcription at TATA-less promoters.

We are entering an era wherein disease mechanisms are being described in molecular terms, and instances of faulty gene expression in the genesis of a disease are becoming apparent. Newer possibilities of controlling gene expression for biomedical applications need to be pursued. An understanding of the Zn finger DNA binding domain of Sp1 and other Zn finger proteins has led to a novel potential application. Klug and coworkers⁵³ have designed a DNA-binding protein comprising three Zn fingers that can bind specifically to an activated oncogene implicated in myeloid leukemias and block its transcription in cultured cells, thus restoring the growth control mechanisms of the cells.

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Prospectives of botanical and microbial products as pesticides of tomorrow

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This article deals with some botanical and microbial products which have demonstrated their efficacy in the management of agricultural pests. Their merits over the existing synthetic pesticides have been discussed.

IN SPITE of the use of all available means of plant protection, about one-third of the yearly harvest of the world is destroyed by pests. Tropical countries, because of their temperature and their particular environment suffer severe losses from pests. Until 1945, the measures used against the pests were mainly based on metallic salts (frequently copper salts) and these were efficient in protecting cultivated crops only from some pest invasions. The discovery and use of DDT in 1940 and then BHC and subsequent development of the chlorinated cyclodienes marked a major advance in the field of crop protection. These chemicals have made great contributions to plant protection but have also raised a number of ecological and medical problems. The environmental problems caused by these xenobiotics have been recognized from time to time as these chemicals lace the food with residues^{1,2}. It has been estimated that hardly 0.1% of the agrochemicals used in crop protection reach the target pest leaving the remaining 99.9% to enter the environment to cause hazards to non-target organisms including humans³.

Effective pest control is no longer a matter of heavy application of pesticides, partly because of rising cost of petroleum-derived products but largely because excessive use of pesticide promotes faster evolution of resistant forms of pests, destroys natural enemies, turns formerly innocuous species into pests, harms other non-

target species and contaminates food^{2,4}. The number of fumigants for insect control are decreasing drastically as problems of insect resistance have intensified and mounting social pressures against the use of toxic chemicals have limited the introduction of new compounds. At present only two fumigants are in common use: methyl bromide and phosphine. Methyl bromide has been identified as a major contributor to ozone depletion, which casts a doubt on its future use in insect control. There have been repeated indications that certain insects have developed resistance to phosphine which is widely used today. It has been recently reported that at least 447 species of insects and mites and 200 species of plant pathogens and 48 species of weeds are now resistant to chemical pesticides. A serious re-evaluation and radical revision of the basic underlying tenets of pest control philosophy has, therefore, taken place in the past 5-10 years and the concept of pest management advocating suppression of pest populations below levels capable of causing economic injury rather than total eradication has emerged⁵⁻⁷.

The presence of organochlorine in the human body and the associated danger of long-term toxicity has resulted in national and international legislation to limit and even to ban the use of some compounds of this group. There is thus an urgent need for control agents which are less toxic to man and more readily degradable.

Basic research for over more than forty years in biology and biochemistry has made it possible to envisage not only how new pesticides may be synthesized but also a completely new approach for the protection of plants using secondary plant products which may be toxic to a specific pest yet harmless to man. There has been a re-