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SPT Genes: Key Players in the Regulation of Transcription, Chromatin Structure and Other Cellular Processes[1]

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Suppressor of Ty (*SPT*) genes were originally identified through a genetic screen for mutations in the yeast *Saccharomyces cerevisiae* that restore gene expression disrupted by the insertion of the transposon Ty. Classic members of the *SPT* gene family, *SPT11*, *SPT12*, and *SPT15*, encode for the histones H2A and H2B, and for TATA-binding protein (TBP), respectively. Over the past few years, molecular complexes and cellular functions in which other *SPT* gene products involve have been discovered through genetic and biochemical studies in yeast and several other organisms: Key regulators of transcription and chromatin structure, such as DSIF, SAGA, and FACT, all contain *SPT* gene products as essential subunits. In addition, accumulating evidence suggests that *SPT* gene products play more diverse roles, including roles in DNA replication, DNA recombination and developmental regulation. Here we review the current understanding of the functions and roles of the *SPT* genes, with special emphasis on the role of *SPT5* in transcript elongation and in neuronal development in vertebrates.

Key words: chromatin, neuronal development, RNA polymerase II, *SPT*, transcription.

Introduction: Suppressor of Ty (*SPT*) genes

Genetic screening of the yeast *Saccharomyces cerevisiae* has been one of the most powerful approaches in the identification of novel genes and their functions. Sometimes, a rather simple screening leads to the identification of a collection of genes which provide an unparalleled starting point to understand complicated cellular processes. One such example is provided by studies on transcription-defective mutants caused by insertion of the transposon Ty, or its long-terminal-repeat (LTR) δ (reviewed in Ref. *1*). Insertion of Ty or δ sequence in the 5′ region of a gene often abolishes or reduces transcription of the adjacent gene. Such inhibition is probably due to the strong transcription signals within the Ty element interfering with those of the adjacent gene. Therefore, an approach to identify factors that are involved in determining which genes are to be transcribed is the selection and identification of secondary mutations that restore the defect in gene expression.

Winston and co-workers have taken this approach and isolated over a dozen genes that they designated Suppressor of Ty (*SPT*) (*1–3*). Certain insertional mutations, located in the 5′ or upstream region of *HIS4* or *LYS2*, abolish expression of these genes, resulting in a His^- or Lys^- auxotrophy. Selection for *spt* mutants, or the Spt^- phenotype, in these strains therefore selects for His^+ or Lys^+ revertants. In all of the *spt* mutants examined, transcription initiating from Ty or δ was reduced, and conversely, normal transcription of the adjacent gene was restored. Therefore, it was deduced that a common role for the *SPT* gene products is to affect promoter function, such that transcription from Ty or δ increases and transcription from the adjacent gene decreases. However, further careful examination of the *spt* mutants has suggested that *SPT* genes play partially overlapping but distinct roles *in vivo* (*2*). *SPT* genes have been classified into different groups based on the discovery of additional mutational phenotypes and of different patterns of suppression of insertional mutations. For example, mutations in *SPT3, SPT7, SPT8,* and *SPT15* cause defects in both mating and sporulation, and mutations in *SPT4, SPT5, SPT6, SPT11,* and *SPT12* strongly suppress solo δ insertional mutations but only weakly suppress Ty insertional mutations. Since the discovery that each of these two groups contains genes for TBP and histones respectively, it has been proposed and in some cases proven, that they exhibit TFIID- and histone-related functions, as described below.

SPT3, SPT7, SPT8, and *SPT15*: TFIID and SAGA

TFIID is a large protein complex that contains TATA-binding protein (TBP) and TBP-associated factors (TAFs) and it plays a central role in promoter recognition by the RNA polymerase II (pol II) transcription machinery (reviewed in Ref. *4*). Although TFIID was initially identified in human HeLa cell extracts (*5*), purification of the human complex was impeded by its tendency to lose activity during the purification process. Instead, active yeast TBP was biochemically purified first, and the gene coding for the protein was cloned and found to be identical to *SPT15* (*6–8*). Later, it was confirmed that TBP from other species, including humans, are encoded by *SPT15* homologues. The identification of Spt15 as TBP supports the notion that *SPT* gene products play a role in specifying which genes are to be transcribed. For more general information on TFIID functions in transcription and chromatin, the reader is

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TABLE I. ***SPT* genes.**

Name	Essential in yeast?	Structure/function
SPT1 = HIR2	no	a repressor component
SPT2 = SIN1	no	HMG1-like, a chromatin component?
SPT3	no	a SAGA component
SPT4	no	metal-finger, a DSIF subunit
SPT5	yes	acidic, a DSIF subunit
SPT6 = SSN20	yes	acidic, nucleosome assembly activity
SPT7	no	a SAGA component
SPT8	no	a SAGA component
SPT10	no	regulates expression of several genes
SPT11 = HTA1	no[a]	Histone H2A
SPT12 = HTB1	no[a]	Histone H2B
SPT13 = GAL11	no	a Mediator component
SPT14	yes	regulates expression of several genes
SPT15	yes	TBP
SPT16 = CDC68	yes	acidic, a FACT subunit
SPT20 = ADA5	no	a SAGA component
SPT21	no	regulates expression of several genes

[a]Since each histone gene is duplicated, deletion of one gene is not lethal.

directed to other extensive reviews (*4*, *9*, *10*).

The discovery that Spt15 in yeast and TBP in other species are homologues has engendered interest and attention in the function of the other *SPT* genes, especially *SPT3*, *SPT7*, and *SPT8*. Initial investigations and data did not support the hypothesis that these genes encode for other TFIID components (*i.e.* TAFs). However, the research was heading in the right direction. Further clues to the function of these genes arrived with the discovery of a new *SPT* gene, *SPT20*, and its identification as *ADA5*, and the fact that the phenotypes of *SPT20/ADA5* mutants were similar to those of *SPT3*, *SPT7*, and *SPT8* (*11*). *ADA* (for adaptor) genes, a genetically-defined group of genes, encode proteins that interact with each other and with another protein, Gcn5. More recently, Gcn5 has been identified as a histone acetyltransferase that is part of the multi-protein complex termed SAGA (Spt-Ada-Gcn5–containing histone acetyltranseferase), which contains Spt3, Spt7, Spt8, Spt20/Ada5, and other Ada proteins (*12*). The SAGA protein complex also contains a subset of TAFs, but not TBP, which suggests a structural convergence between TFIID and SAGA (*13*). A possible human counterpart of SAGA, the holo-P/CAF (P300/CBP-associated factor) complex, also contains human homologues of the Spts, TAFs, and TAF-related proteins (*14*). Genome-wide analyses in yeast support the idea that TFIID and SAGA play redundant roles *in vivo* (*15*). Several reports indicate that SAGA and P/CAF complexes in part regulate transcription through the binding and acetylation of nucleosomes and other transcription factors (*16–18*).

SPT4, *SPT5*, *SPT6*, *SPT11*, and *SPT12*: The histone group

The second group of *SPT* genes was named after two of its members (*SPT11* and *SPT12*) that encode for histones H2A and H2B. Each of the four histone genes (H2A, H2B, H3, and H4) are duplicated in yeast and therefore deletion of one copy of a gene is not lethal. However, disruption of histone genes, or overproduction of histones, causes changes in nucleosome patterns and changes in the level of transcription at several loci, including at Ty insertion sites (*1*). This is one of the earliest illustrations and confirmations of the widely accepted idea that chromatin regulates gene expression. Further evidence of the role for the "histone group" of genes was derived from studies on the genes for *SWI* (for switch) and *SNF* (for sucrose non-fermenting). Swi/Snf is a well-characterized protein complex capable of remodeling chromatin in an ATP-dependent manner (reviewed in Ref. *19*). A genetic screen for suppressors of *swi2/snf2* led to the discovery of *SSN20* (for suppressor of *snf2*), which is identical to *SPT6* (*3*). Later research has demonstrated that mutations in *SPT4*, *SPT5*, *SPT6*, *SPT11*, *SPT12*, and *SPT16* also suppress *snf2/swi2*, and in most cases, *snf5* and *snf6*. More recent genetic and biochemical studies substantiate the connection between these genes, and chromatin and transcription. This connection is discussed in more detail below.

Spt6 appears to interact directly with histones to regulate chromatin structure (*20*). This interaction is mediated by the globular domain of histone H3, which is different from the N-terminal tail that binds other proteins such as Sir3-Sir4 and Tup1. Remarkably, Spt6 alone is capable of nucleosome assembly in an ATP-independent manner *in vitro*. Spt6 is a large protein with a cluster of acidic amino acids, and may function by using a similar mechanism to the histone chaperones such as nucleoplasmin and nucleosome assembly protein (NAP)–1. These proteins contain acidic regions that interact with basic histones to assemble nucleosomes (*21*, *22*).

A number of genetic studies indicate that *SPT4*, *SPT5*, and *SPT6* are involved in a common regulatory pathway (*23*, *24*; reviewed in Ref. *1*). However, recent biochemical studies indicate that Spt4 and Spt5 form a molecular complex that does not contain Spt6 (*24*, *25*). The Spt4-Spt5 complex, and its human counterpart [DRB sensitivity-inducing factor (DSIF)], are considered to regulate transcript elongation through pol II, as described in the next section. Despite this biochemical separation of Spt4-Spt5 and Spt6, transcript elongation *in vivo* needs to overcome nucleosomal blocks, and it has been demonstrated that Spt16 is involved in this regulation (*26*). Further discussion below helps provide a more integral view of the "histone group" genes.

Roles for Spt4-Spt5/DSIF in transcript elongation

Pol II elongation is a highly regulated process that involves *cis*-acting elements, mainly located on nascent RNA, and *trans*-acting factors, some of which have properties expected of general elongation factors. Included in these factors and elements are TFIIF, Elongin, and ELL, which have the *in vitro* function of expediting elongation by suppressing pause or arrest of pol II through direct interaction with the elongation complex (reviewed in Refs. *27* and *28*). Recent studies using 5,6-dichloro-1-β-D-ribofuranosylbenzimidazole (DRB), a synthetic inhibitor of mRNA synthesis, have led to the identification of additional novel elongation factors, namely DSIF, negative elongation factor (NELF) and positive transcription elongation factor (P-TEF) b (*28–30*), which together play a role in a critical control point during pol II elongation.

DSIF is a heterodimer composed of Spt5 and Spt4 and is capable of both stimulating and repressing pol II elongation. The exact mechanism by which DSIF has opposing functions is not clear, however the identification and presence of multiple proteins that interact with DSIF may provide an answer to this question.

When playing a repressive role, DSIF acts together with

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NELF and P-TEFb in the DRB-sensitive control of pol II elongation. Following transcription initiation, pol II moves downstream of the promoters and enters into the productive elongation step. In the presence of DRB, however, pol II pauses about 30 nt downstream of the transcription start site (*25*). Biochemical analyses suggest that DSIF, together with NELF, directly bind pol II to repress elongation, while P-TEFb, a cyclin-Cdk pair with DRB-sensitive protein kinase activity, reverses the repression by phosphorylating the C-terminal domain (CTD) of the pol II largest subunit (Fig. 1A) (*31*, *32*). This agrees with the suggested role for the pol II CTD, which is reversibly phosphorylated during the transcription cycle and its hyperphosphorylated form is found in actively transcribed regions of genes (*33*). This domain is therefore considered to play positive roles in pol II elongation as well as in mRNA processing. The DRB-sensitive elongation step that involves DSIF, NELF, and P-TEFb is probably a general rate-limiting step during pol II transcription (*29*) because DRB affects most, if not all, cellular mRNAs (*34*).

In transcription assays using crude nuclear extracts, DSIF does not repress transcription without DRB, because endogenous P-TEFb efficiently reverses the repression. Instead, DSIF can stimulate elongation under certain conditions *in vitro*. Limiting NTP concentrations (*25*) or using a template that produces long transcripts (*35*), both of which increase the frequency or the number of pauses that pol II encounters during elongation, uncovers the stimulatory activity of DSIF. DSIF thus appears to increase the ability of pol II to bypass pausing that occurs by chance.

Studies on human immunodeficiency virus (HIV) transcription have revealed the possible mechanism underlying the stimulatory activity of DSIF. Successful transcription from the HIV LTR promoter requires the viral transactivator Tat (*36*, *37*). In its absence, pol II encounters a strong block to elongation at around +50 from the transcriptional start site. Tat binds to an RNA element called TAR (for Tat-responsible element) on the nascent transcript, and removes the block by mechanisms that are still not fully understood. To date, several cellular proteins, including P-TEFb, Tat-stimulatory factor (SF)–1, and the Spt5 subunit of DSIF, have been independently identified as factors that are required for Tat-dependent transcription (*38–41*). More recent work has demonstrated that Tat-SF1, Spt5, and possibly P-TEFb are part of a large protein complex that binds to both Tat and pol II and mediates Tat-dependent transcription (Fig. 1B) (*42*, *43*). Though Tat-SF1 was originally identified as a Tat-specific cofactor, some evidence has suggested that it plays a more general role in pol II elongation (*43*, *44*). Thus, DSIF may exert its positive effect as part of this multi-protein complex.

In vivo evidence supporting the role for DSIF in pol II elongation has come from studies in yeast and fly. In *S. cerevisiae*, some *spt4* and *spt5* mutations render yeast sensitive to the drug 6-azauracil, which reduces intracellular NTP concentrations and thus is expected to decrease the rate of transcript elongation (*24*). In addition, some phenotypes with *spt4* and *spt5* mutations are either suppressed or enhanced by mutations in two of the largest subunits of pol II and in elongation factor TFIIS. In *Drosophila melanogaster*, immunostaining of its polytene chromosome showed that Spt5 mainly co-localizes with the transcriptionally-active, phosphorylated form of pol II and it is also recruited to active chromosomal sites during development or upon extracellular stimuli (*45*, *46*). These findings indicate a positive role for Spt5 in transcription. However, more detailed studies of the three heat shock protein (*hsp*) gene loci give support to the proposed dual roles of DSIF in pol II elongation (*46–48*). Transcription of the three *hsp* genes is induced by heat or other types of stress. In the unstressed

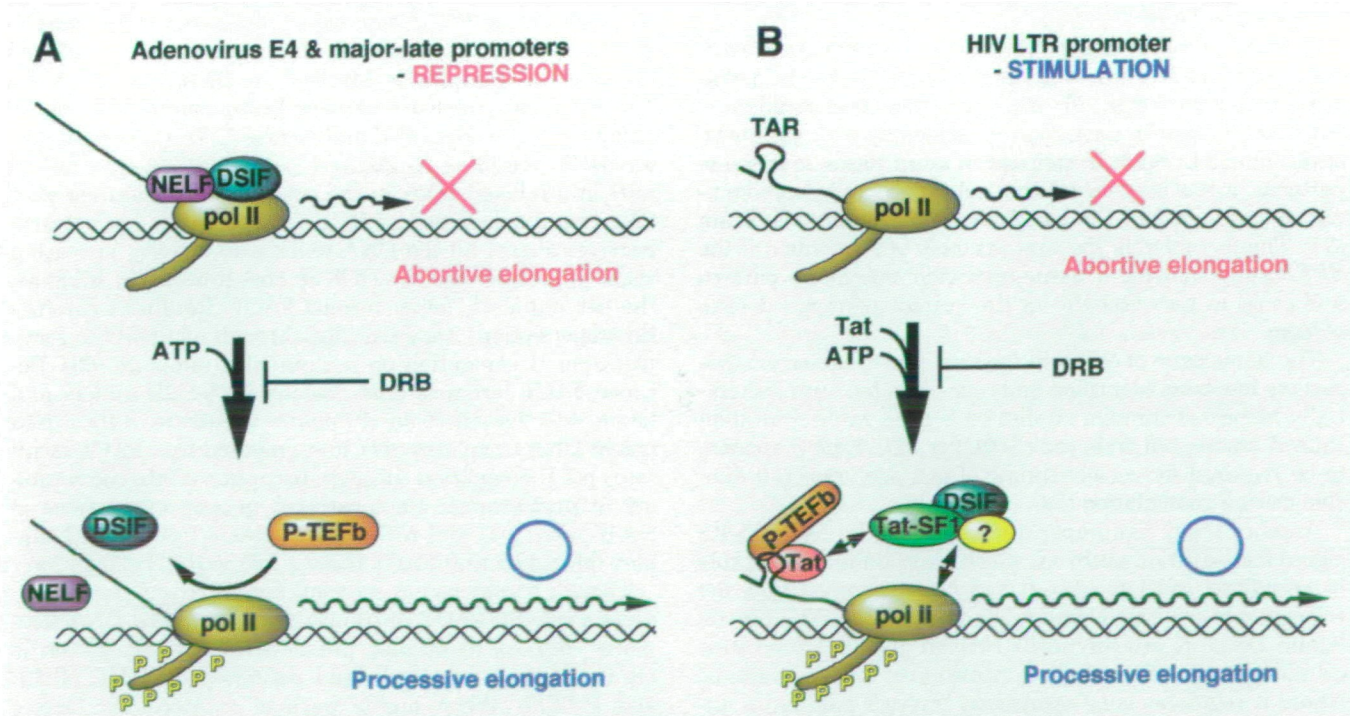

Fig. 1. Illustration of the dual roles of DSIF/Spt4-Spt5 in elongation by RNA polymerase II.

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state, the unphosphorylated form of pol II and Spt5 exclusively localize at the 5′ end of these genes. Upon heat shock, several factors including P-TEFb are recruited onto these genes. As a consequence, pol II becomes phosphorylated, and pol II and Spt5 can be localized further downstream of these genes. Interestingly, this type of promoter-proximal pausing by pol II is observed not only in the inducible *hsp* genes but also in the constitutively-expressed *β-1 tubulin* gene (*49*), and the developmentally-regulated *c-myc* gene (*50*). This suggests that a broad range of genes might be regulated immediately downstream of the promoters, most likely through the actions of DSIF, NELF, and P-TEFb.

Developmental regulation by Spt5 and Spt6

Genetic studies using zebrafish (*Danio rerio*) and nematodes (*Caenorhabditis elegans*) have shed light on the possible roles that *SPT* genes play during the development of multi-cellular organisms.

In the vertebrate brain, several distinct classes of neurons are generated from common progenitor cells. Generation of mutants in the zebrafish that affect neuronal development has resulted in a collection of various phenotypes (*51*). One of the mutants named foggy, which displays a reduction of dopamine-containing neurons and a corresponding surplus of serotonin-containing neurons, was found to be caused by a missense mutation of the zebrafish *SPT5* homologue (*35*). This mutation results in a single amino acid substitution at the C-terminal region of Spt5. The C-terminal region is well conserved across a broad range of species but its function in the mature protein is not known. *In vitro* experiments show that this mutation abolishes the repressive, but not stimulatory, activity of DSIF on pol II elongation. The *in vivo* and *in vitro* evidence suggests that alteration in neuronal cell fates could be caused by disruption of DSIF repressive activity on elongation through ectopic expression of genes that are normally repressed by DSIF.

Spt5 is a protein that is ubiquitously expressed in various human adult tissues (*52*) and is considered to be a general elongation factor (*45*, *46*). A question that remains is why such a specific phenotype is caused by a single nucleotide change in Spt5. In contrast to adult tissue expression patterns, it has been found that zebrafish Spt5 is predominantly expressed in the early-developing embryonic brain (*35*). This is probably the first example of a member of the *SPT* family showing a tissue-restricted expression pattern and could in part account for the specific neuronal defects of foggy.

The homologue of *SPT6* in the nematode *Caenorhabditis elegans* has been identified and named emb-5, and was initially identified through studies on a gene whose mutation caused conditional embryonic lethality (*53*). Emb-5 appears to be required for correct timing of gut precursor cell division during gastrulation (*54*).

Another study has implicated emb-5 in the Notch-Delta signal transduction pathway, which plays an important role in specifying cell fate (*55*). It has been proposed that the transmembrane-type receptor Notch, upon activation by its ligand Delta, is proteolytically cleaved and that the intracellular domain of Notch then translocates into the nucleus, where it regulates gene expression through binding to nuclear proteins (reviewed in Ref. *56*). Emb-5 has been identified as interacting with the intracellular domain of the *C. elegans* Notch homologue. Genetic data has suggested that EMB-5 has a positive downstream role in Notch-mediated signaling.

Roles for Spt4 and Spt6 in recombination and chromosomal segregation

Genetic studies in yeast suggest that Spt4 and Spt6 are also involved in DNA recombination and chromosomal segregation (*57–59*). Some mutations in *SPT4* or *SPT6* show a hyper-recombination phenotype that enhances a broad range of recombination events, such as gene conversions and deletions between direct repeats (*57*). Mutants for *spt4* or *spt6* also show chromosomal mis-segregation, probably due to functional defects in the centromere-kinetocore complexes (*58*). It is not clear whether Spt4 and Spt6 are directly involved in these processes, or affect the processes indirectly through regulation of transcription and/or chromatin structure.

Roles for Spt16 in transcription and replication

SPT16, also known as *CDC68*, is genetically related to the "histone group" of *SPT* genes. Spt16 is believed to function by forming a complex with Pob3 in yeast, and its putative homologue SSRP1 in vertebrates. This heterodimeric complex has been independently identified using three different biochemical strategies and it has been implicated in both transcription and replication. In *S. cerevisiae*, the CP (Cdc68-Pob3) complex was identified as a protein complex that bound to a DNA-polymerase α-affinity column (*60*). Some mutations in *SPT16/CDC68* cause arrest at the G1 phase of the cell cycle and, when combined with mutations in *POL1* gene (coding for the catalytic subunit of DNA polymerase α), display severe growth defects. In addition, mutations in *POB3* show the Spt$^-$ phenotype, render yeast sensitivity to the dNTP synthesis inhibitor hydroxyurea, and display severe synthetic defects with mutations in *SPT16/CDC68*, *POL1*, and other genes coding for replication factors (*61*). These genetic data support the hypothesis that the CP complex is involved in DNA replication. In *Xenopus laevis*, the heterodimeric factor comprised of Spt16 and SSRP1 was identified and termed DNA unwinding factor (DUF) based on its ability to introduce negative supercoils into relaxed DNA in the presence of topoisomerase I (*62*). Immunodepletion of DUF from *Xenopus* egg extracts removes almost all the DNA replication activity, indicating some important role for DUF in this process. In humans, the heterodimeric factor termed FACT (facilitates chromatin transcription) was identified through its ability to stimulate pol II elongation on a chromatin template (*63*). Because FACT interacts with histone H2A-H2B dimers and has a selective effect on chromatin templates rather than naked DNA templates (*64*), it is proposed that FACT facilitates pol II elongation through disruption of histone octamers in nucleosomes. Demonstrated genetic interactions of Spt16 with Spt4 and with the elongation factor TFIIS support the notion that FACT plays a role in pol II elongation.

Though it may sound contradictory, a recent report suggests a role for FACT in transcription on naked DNA templates (*65*). As mentioned previously, pol II elongation is regulated both negatively and positively by DSIF, NELF and P-TEFb. When highly purified transcription factors and naked DNA templates are used in transcription as-

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says, P-TEFb is ineffective in suppressing DSIF/NELF inhibition, and further addition of FACT fully reverses the inhibition. Therefore it appears that FACT removes the block imposed by histones, DSIF and NELF on elongation.

Other *SPT* genes

SPT2 is identical to *SIN1* (for switch independent), which was isolated in a screen for suppressor mutations of *swi1* mutants. *SPT2/SIN1* encodes for an HMG1-like protein that genetically interacts with the pol II CTD (*66*), histones H3 and H4 (*67*), and several regulators of chromatin structure. Although Spt2/Sin1 has been proposed to be a component of chromatin, its exact role is yet to be determined.

SPT13 is identical to *GAL11*, which is a gene required for efficient utilization of galactose and for the Gal4-mediated expression of *GAL1*, *GAL7*, and *GAL10*. Spt13/Gal11 is now known to be a component of the Mediator complex that binds to the pol II CTD and potentiates both basal and activator-dependent transcription (*68*). Spt13/Gal11 physically associates with Gal4, thereby mediating a transactivation signal to the basal transcription machinery (*69*, *70*).

SPT1 is identical to *HIR2* (histone regulatory) (*71*). Spt1/Hir2 and another protein (Hir1) form a repressor complex that affects expression of histone genes (*72*). Potentially, this complex is also involved in silencing at telomeres and at the *HM* loci (*73*). Mutations in two other *SPT* genes, *SPT10* and *SPT21*, confer similar phenotypes to mutations in *SPT1* (*71*). These gene products also appear to be involved in histone gene expression (*74*).

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