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Genetics of Eukaryotic RNA Polymerases I, II, and III

JACQUES ARCHAMBAULT AND JAMES D. FRIESEN*

Department of Genetics, The Hospital for Sick Children, 555 University Avenue, Toronto, Ontario M5G 1X8,[1] and the Department of Molecular and Medical Genetics, University of Toronto, Toronto, Ontario, M5S 1A8,[2] Canada*

* Corresponding author.

INTRODUCTION

Recent studies of nuclear DNA-dependent RNA polymerases (RNAP) in eukaryotic organisms provide an instructive example of how the combination of genetics and biochemistry can propel the rapid expansion of biological knowledge. Prior to 1984, biochemists had succeeded in purifying three RNAPs, termed RNAPI, RNAPII, and RNAPIII or RNAPA, RNAPB, and RNAPC, respectively, from a variety of eukaryotic sources. These three RNAPs differ in their nuclear location, chromatographic behavior, salt requirements, subunit composition, sensitivity to the toxin α-amanitin, and, most importantly, the class of RNA they synthesize: RNAPI synthesizes the rRNA precursor, RNAPII transcribes all the protein-encoding genes and certain small nuclear RNA genes, while RNAPIII is responsible mainly for the synthesis of 5S RNA and tRNAs. Despite these differences, however, all three RNAPs were found to share a common subunit structure which has been conserved across a wide spectrum of organisms.

These findings raised many questions, many of which are answerable only through the collaboration of biochemistry and genetics. Are all of the polypeptides that copurify with RNAP activity bona fide subunits; i.e., are they essential for the structure and/or function of the enzyme? What is the function of each subunit? What is the pathway of their assembly? With which other subunits does each interact? Which subunits bind to transcription factors, to substrates, or to DNA? Precisely which regions of the RNAP subunits are conserved, and what is their role?

Many of these questions remain to be answered, but in some cases genetic techniques have begun to free their answers from some of the practical bounds inherent in a strictly biochemical approach. These limitations include the inability to reconstitute, in vitro, eukaryotic RNAPs from subunits in whole or in part, the impracticality of determining the complete amino acid sequence of large proteins, and the impossibility of introducing permanent variations into RNAP components.

On the other hand, a solely classical genetic approach to RNAP studies is also severely limited. Only one inhibitor of RNAP activity, the fungal toxin α-amanitin, has been used successfully to isolate mutant forms of RNAP. However, this approach has been limited to organisms that are sensitive to the drug and has yielded only a narrow spectrum of mutations; all α-amanitin resistance mutations affect the largest subunit of RNAPII. Attempts to select or screen for conditional-lethal RNAP mutations in eukaryotes have met with little success.

Cloned genes encoding subunits of RNAPs have become the centerpiece of current studies of eukaryotic RNAPs. Primarily in *Saccharomyces cerevisiae*, *Drosophila melanogaster*, and *Caenorhabditis elegans*, but with applicability to other organisms, the availability of cloned genes immediately allowed the determination of the primary structure of RNAP subunits, the establishment of whether individual subunits are essential for cell viability, the generation of random and targeted mutations which are useful for further investigations of function, assembly and interaction, and the production of large-scale amounts of individual subunits. Already the identification of mutations through the use of cloned subunit genes has proven invaluable in determining particular regions of subunits that lead to defects in transcription start site selection, initiation, elongation, termination, and interaction with regulatory factors. We can look forward to the further application of both traditional and molecular genetics in defining interactions among subunits, among regions of subunits, and between subunits and regulatory factors. In the future these will increasingly be related to aspects of physiology in both the cell and the whole organism and also to complex biological processes such as development and cellular differentiation.

This review will focus on the effects of mutations in genes encoding subunits of RNAPI, RNAPII, and RNAPIII and will only briefly summarize what is known about the general structure of the RNAPs. Detailed information about the structure and biochemical properties of eukaryotic RNAPs can be found in some recent reviews on all three RNAPs (127, 160, 161) or on RNAPII (30, 151, 153, 191) or RNAPIII (51, 52).

The mutations referred to in this review are summarized in Tables 1 and 2.

Genetic Nomenclature

The nomenclature used for describing genes encoding subunits of eukaryotic RNAPs is not yet standardized. This is exemplified by the situation in the yeast *S. cerevisiae*, in which three different systems of nomenclature exist. Because many of the mutations reviewed here have been isolated and characterized in *S. cerevisiae*, a brief explanation of these three nomenclature systems is presented.

In the first system (192), a gene is named *RPA*, *RPB*, or *RPC* to indicate whether it was first isolated as a gene encoding a subunit of RNAPI (A), RNAPII (B), or RNAPIII (C), followed by a number that ranks the subunit by size relative to that of the others (for example, the largest subunit is given the number 1, the second-largest subunit is given the number 2, etc.). In this system, the gene encoding the largest subunit of RNAPII is known as *RPB1*.

In the second system (75), *RPO* (for RNA polymerase) is followed by the number 1, 2, or 3 to indicate whether it was identified as a gene encoding a subunit of RNAPI, RNAPII, or RNAPIII, respectively, followed by another number which refers to the rank of the subunit, by size, relative to the others. Using this system, the gene encoding the largest subunit of RNAPII is known as *RPO21*.

In the third system (143), *RPA*, *RPB*, or *RPC* is followed by a number referring to the molecular mass of the subunit (in kilodaltons). In this system, the gene encoding the largest subunit of RNAPII is known as *RPB220*.

In all three systems of nomenclature, the name of a gene encoding a subunit that is common to two or to all three RNAPs refers to the RNAP for which this particular subunit gene was first identified. For example, the gene *RPO26* (or *RPB6*) was isolated as encoding the sixth-largest subunit of RNAPII. This subunit is also found in RNAPI (8th largest) and RNAPIII (11th largest).

The situation is further complicated when one considers designating mutant alleles. In conventional *S. cerevisiae* genetic nomenclature, a mutant allele is given a number that is separated from the name of the gene by a hyphen. The problem arises when two different alleles are given the same number but use a different gene designation; for example, *rpb1-1* and *rpo21-1* represent two different alleles of the same gene. It is therefore impossible to unify all three systems of nomenclature without having to renumber most of the mutant alleles that have already been isolated. For this reason, all three systems will be used in this review.

TABLE 1. Summary of selected mutations affecting the largest subunits of RNAPI, RNAPII, or RNAPIII

Allele[a]	Subunit	RNAP	C.R.[b]	Amino acid substitution or insertion[c]	Organism[d]	Phenotype(s)[e]	Reference(s)[f]
rpa190-1	RPA190	I	A	G77D	S.c.	ts(1×), wt(Mx) assembly/stability	184
rpa190-2	RPA190	I		W679Stop	S.c.	ts, strain probably contains a tRNA suppressor	184
rpa190-3	RPA190	I		G728D	S.c.	ts	184
rpa190-5	RPA190	I	A	H75Y	S.c.	ts, assembly/stability	184
rpo21-1	RPO21	II	A	G79D	S.c.	ts, assembly/stability	65
rpo21-2	RPO21	II	A, C	G111D, E360K	S.c.	ts, assembly/stability	65
rpo21-3	RPO21	II	A	G111D	S.c.	ts, assembly/stability	65
rpo21-4	RPO21	II		W954(LELE)P	S.c.	ts, suppressed by high dosage of *RPO26*	7, 10
rpo21-5	RPO21	II		Q124(ARAR)A	S.c.	ts	7
rpo21-6	RPO21	II		R1135(LELE)S	S.c.	ts, sensitivity to 6AU, suppressed by high dosage of *PPR2*	7, 8
rpo21-7	RPO21	II		T1141(SSSS)T	S.c.	ts, sensitivity to 6AU, suppressed by high dosage of *PPR2*	7, 8
rpo21-8	RPO21	II		V32(ARAR)A	S.c.	ts	7
rpo21-17	RPO21	II		V1107(ARAR)A	S.c.	ts, sensitivity to 6AU, suppressed by high dosage of *PPR2*	7, 8
rpo21-18	RPO21	II		L1236(TRARV)I	S.c.	ts, sensitivity to 6AU, suppressed by high dosage of *PPR2*	7, 8
rpo21-19	RPO21	II		D1257(SSSS)H	S.c.	Sensitivity to 6AU, suppressed by high dosage of *PPR2*	7, 8
rpo21-23	RPO21			E(1230)K, E(1264)K	S.c.	ts, sensitivity to 6AU, suppressed by high dosage of *PPR2*	8
rpo21-24	RPO21	II		E(1230)K	S.c.	ts, sensitivity to 6AU, suppressed by high dosage of *PPR2*	8
rpb1-1	RPO21	II	H	G1437D	S.c.	ts, ceases mRNA synthesis rapidly	130
rpb1-5	RPO21	II	C	R335C	S.c.	ts, not assembly defect	90
rpb1-9	RPO21	II		P24S	S.c.	ts, not assembly defect	90
rpb1-10	RPO21	II	F	G558D, M818I	S.c.	ts, not assembly defect	90
rpb1-13	RPO21	II		P172S	S.c.	ts, assembly	90
rpb1-14	RPO21	II		R412H	S.c.	ts, cs, not assembly defect	90
rpb1-15	RPO21	II		I1327N	S.c.	ts, cs, assembly	90
rpb1-17	RPO21	II	D	M487I	S.c.	ts, cs, not assembly defect	155
rpb1-18	RPO21	II		G166S	S.c.	ts, assembly	90
rpb1-19	RPO21	II		C1240Y	S.c.	ts, assembly	90
rpb1-501	RPO21	II	G	S1096F	S.c.	Suppressor of Ty insertions, start site selection	64
rpb1-502	RPO21	II	G	T1080I	S.c.	Suppressor of Ty insertions, start site selection	64
rpb1-510	RPO21	II	G	E1061A	S.c.	TR, suppressor of *rpb2-2*	111
rpb1-511	RPO21	II	C	R326C	S.c.	TR, suppressor of *rpb2-2*	111
rpb1-513	RPO21	II	H	F1410L	S.c.	TR, suppressor of *rpb2-2*	111
rpb1-514	RPO21	II		S1449L	S.c.	TR, suppressor of *rpb2-2*	111
rpb1-551	RPO21	II	H	V1428F	S.c.	TR, suppressor of CTD deletion	132
rpoB1	RPO21	II			S.c.	Dissociation of RPB4/RPB7, initiation, elongation	150
sit1	RPO21	II	F		S.c.	Suppressor of *GCN4*, *BAS1*, *BAS2* triple deletion	11
A21	RPII215	II	F	N792D	Mouse	Ama^r	13
C4	RpII215	II			D.m.	Ama^r, decreased rate of elongation in vitro	32
WJK1	RpII215	II			D.m.	ts lethal, premature termination	119
Ubl	RpII215	II			D.m.	Recessive lethal, dominant phenotype similar to *Ubx*	123
m118m526	ama1	II			C.e.	"Super" Ama^r; 20,000× more resistant to α-amanitin	146
rpc160-103	RPC160	III	A	H80Q, K85I	S.c.	Null	179
rpc160-106	RPC160	III	A	F96L	S.c.	wt	179
rpc160-114	RPC160	III	A	K108R	S.c.	ts	179
rpc160-115	RPC160	III	A	H83L	S.c.	wt	179
rpc160-124	RPC160	III	A	A75Δ, S76Δ	S.c.	Null	179
rpc160-126	RPC160	III	A	H78Q	S.c.	wt	179
rpc160-134	RPC160	III	A	T69N	S.c.	ts, unstable in vitro, dissociation of C82, C34, and C31	179
rpc160-168	RPC160	III	A	C67H, C70H	S.c.	Null	179

Continued on following page

TABLE 1—*Continued*

Allele[a]	Subunit	RNAP	C.R.[b]	Amino acid substitution or insertion[c]	Organism[d]	Phenotype(s)[e]	Reference(s)[f]
rpc160-169	RPC160	III	A	C67H, C70Q	S.c.	Null	179
rpc160-176	RPC160	III	A	C107H, C110Q	S.c.	Null	179
rpc160-177	RPC160	III	A	C107H, C110H	S.c.	Null	179
rpc160-178	RPC160	III	A	C107Q, C110H	S.c.	Null	179
rpc160-179	RPC160	III	A	H80Q	S.c.	Null	179
rpc160-180	RPC160	III	A	C67Q, C70H	S.c.	Null	179
rpc160-188	RPC160	III	A	C67Q, C70Q	S.c.	Null	179
rpc160-189	RPC160	III	A	H80L	S.c.	Null	179
rpc160-213	RPC160	III	A	C77H	S.c.	Null	179
rpc160-214	RPC160	III	A	H78Q, H83L	S.c.	wt	179
rpc160-215	RPC160	III	A	H80Y	S.c.	Null, analogous to *rpa190-5*	179
rpc160-220	RPC160	III	A	C77Q	S.c.	Null	179
rpc160-223	RPC160	III	A	H80C	S.c.	Null	179
rpc160-226	RPC160	III	A	C67A	S.c.	Null	179
rpc160-227	RPC160	III	A	C70A C77Δ, H78Δ, I102R	S.c.	Null	179
rpc160-228	RPC160	III	A	G79D	S.c.	Null, analogous to *rpo21-1*	179
rpc160-229	RPC160	III	A	G82D	S.c.	Null, analogous to *rpa190-1*	179
rpc160-230	RPC160	III	A	C107A	S.c.	Null	179
rpc160-231	RPC160	III	A	C110A	S.c.	Null	179
rpc160-232	RPC160	III	A	C67Q, C77A, H80R	S.c.	Null	179
rpc160-233	RPC160	III	A	C77A, H80R, C107Q, C110Q	S.c.	Null	179
rpc160-234	RPC160	III	A	C77A, H80R	S.c.	Null	179
rpc160-235	RPC160	III	A	C77A, H78R, H80R, H83R	S.c.	Null	179

[a] Not all mutations have been given an allele designation.
[b] C.R., conserved region as diagrammed in Fig. 1 and 2. Other than the two cysteine pairs, residues in this region have not been highly conserved.
[c] For linker insertion mutations, the predicted amino acids inserted are in parentheses.
[d] Organisms: S.c., *S. cerevisiae*; E.c., *E. coli*; D.m., *D. melanogaster*; C.e., *C. elegans*.
[e] Phenotypes: ts, recessive temperature sensitivity; cs, recessive cold sensitivity; TR, dominant temperature resistance; Ama^r, resistance to α-amanitin; wt, wild type. For some alleles, the phenotype varies according to the copy number: 1×, one copy; M×, multiple copies.
[f] Most recent reference.

General Structure

The polypeptide compositions of purified eukaryotic RNAPI, RNAPII, and RNAPIII are very similar. All three enzymes are multisubunit complexes containing two large polypeptides that are homologous to the two largest subunits (β and β′) of the *Escherichia coli* enzyme and are associated with several smaller subunits. Some of the small subunits are common to all three enzymes, whereas others are common to only RNAPI and RNAPIII. Since RNAPI, RNAPII, and RNAPIII are best characterized in *S. cerevisiae*, the structures of the yeast enzymes are summarized in Tables 3 through 5.

The overall morphology of RNAP from *E. coli*, as well as that of *S. cerevisiae* RNAPI and II, has been investigated by electron microscopy of two-dimensional crystals (34, 35, 157). In all three enzymes, a channel of approximately 25 Å (2.5 nm) in diameter was found that is similar to the proposed DNA-binding domain of DNA polymerase (34, 35, 135, 157). It might be significant that a stretch of amino acid residues similar to those forming the DNA-binding region of DNA polymerase I has been observed in the largest subunit of *E. coli* and eukaryotic RNAPs (3). These observations are consistent with biochemical experiments (reviewed in reference 142) that suggest a role for the largest subunit of *E. coli* and eukaryotic RNAPs in binding to DNA. A second channel was observed in the more highly resolved structure of *S. cerevisiae* RNAPII, which is wide enough to accommodate single-stranded nucleic acids, perhaps the nascent RNA (34).

Sequence Conservation of Individual Subunits

In addition to the conservation of their basic subunit composition described above, RNAPs are also related at the level of individual subunits. This was first demonstrated by the observation that antiserum directed against individual subunits could detect subunits of other RNAPs from different organisms (reviewed in references 160 and 161). This conservation of antigenic determinants was confirmed by the isolation and nucleotide sequencing of genes encoding subunits of RNAPs from various sources. Amino acid sequence alignment of the largest subunits of RNAPI, RNAPII, and RNAPIII of eukaryotes as well as that of eubacteria (*E. coli*) and archaebacteria (15, 101, 139) revealed several colinear regions of sequence similarity (3, 83, 118) termed conserved regions A to H (83) (Fig. 1). Furthermore, nine regions of sequence similarity were noted among the second-largest subunit of the prokaryotic and eukaryotic enzymes and were termed regions A to I (46, 172) (Fig. 1). More extensive sequence alignment further refined the boundaries of these 9 regions of sequence similarity into 17 regions, 13 of which are conserved in eubacterial and chloroplast RNAPs (80).

The extensive sequence conservation observed between the two largest subunits of eukaryotic and prokaryotic RNAPs most probably underlies a conservation of their function. Together, these two subunits form the catalytic center of the enzyme. Biochemical experiments have suggested that they are involved in binding the DNA and the nascent RNA (reviewed in reference 153). In addition, the

TABLE 2. Summary of selected mutations affecting the second or third largest subunits of RNAPI, RNAPII, or RNAPIII

Allele[a]	Subunit	RNAP	C.R.[b]	Amino acid substitution	Organism[c]	Phenotype(s)[d]	Reference[e]
Collection	β		D, F, I		E.c.	Increased or decreased termination	92
rpoB8	β		D	Q513P	E.c.	Increased K_m for purines, decreased elongation rate	82
	β		H	K1065R	E.c.	Dominant-negative, nucleotide cross-linking site	85
	β		I	H1237A	E.c.	Reduced promoter clearance	129
	β		F	E813K	E.c.	Dominant-negative, reduced promoter clearance	97
ack1-1	β		F		E.c.	Reduced transcriptional accuracy	105
rpb2-1	RPB2	II		G1142D	S.c.	ts, cs, not assembly defect	90
rpb2-3	RPB2	II		R1106K	S.c.	ts, cs, not assembly defect	90
rpb2-4	RPB2	II		A1016T	S.c.	ts, cs, not assembly defect	90
rpb2-5	RPB2	II		G1068E	S.c.	ts, not assembly defect	90
rpb2-6	RPB2	II		R857K	S.c.	ts, assembly	90
rpb2-7	RPB2	II			S.c.	ts, cs, not assembly defect	90
rpb2-8	RPB2	II		C1188Y	S.c.	ts, cs, not assembly defect	90
rpb2-9	RPB2	II		G913D	S.c.	ts, cs, leaky assembly defect	90
rpb2-10	RPB2	II		P1018S	S.c.	cs, not assembly defect	90
rpb2-11	RPB2	II		C829Y	S.c.	ts, cs, not assembly defect	90
rpb2-12	RPB2	II			S.c.	ts, not assembly defect	90
rpb2-503	RPB2	II		G369D	S.c.	Suppressor of Ty insertions, start site selection	64
rpb2-504	RPB2	II		E368K	S.c.	Suppressor of Ty insertions, start site selection	64
rpb2-510	RPB2	II	I	S1145 L	S.c.	TR, suppressor of *rpb1-1*	111
rpb2-513	RPB2	II	I	D1125N	S.c.	TR, suppressor of *rpb1-1*	111
sit2	RPB2	II			S.c.	Suppressor of *GCN4*, *BAS1*, *BAS2* triple deletion	11
	RPB2	II	ZBR	C1163A	S.c.	Lethal, assembly/stability, reduced zinc binding	175
	RPB2	II	ZBR	I1165C1166GVA	S.c.	Lethal, assembly/stability, reduced zinc binding	175
	RPB2	II	ZBR	C1166S	S.c.	ts(1×), wt(Mx), assembly/stability, reduced zinc binding	175
	RPB2	II	ZBR	G1167F	S.c.	ts(1×), wt(Mx), assembly/stability	175
	RPB2	II	ZBR	L1168P	S.c.	Lethal, assembly/stability, reduced zinc binding	175
	RPB2	II	ZBR	C1182A	S.c.	ts(Mx), assembly/stability, reduced zinc binding	175
	RPB2	II	ZBR	C1185A	S.c.	ts(Mx), assembly/stability, reduced zinc binding	175
SRP3-1	RPA135	I	ZBR	C1127R	S.c.	Suppressor of *rpa190-1*, *rpa190-5*	190
ret1-1	RET1	III		T311K	S.c.	Decreased termination at weak terminators	80
wimp	RpII140	II			D.m.	Recessive lethal, dominant maternal effect	136
rpoA112	α			R45C	E.c.	Assembly	69
rpb3-1	RPB3	II		A30D, G175A	S.c.	ts, assembly	90

[a] Not all mutations have been given an allele designation.
[b] Conserved region as diagrammed in Fig. 1 and 2. ZBR refers to the zinc-binding region of the second-largest subunit. Other than the two cysteine pairs, residues in this region have not been highly conserved.
[c] Organisms: S.c., *S. cerevisiae*; E.c., *E. coli*; D.m., *D. melanogaster*.
[d] Phenotypes: ts, recessive temperature sensitivity; cs, recessive cold sensitivity; TR, dominant temperature resistance; Ama^r, resistance to α-amanitin. For some alleles, the phenotype varies according to the copy number: 1×, one copy; Mx, multiple copies.
[e] Most recent reference.

second-largest subunit is probably involved in binding the substrate nucleotide (reviewed in reference 153; also see below).

Sequence conservation also extends to the smaller subunits. In *S. cerevisiae*, sequences similar to a segment of the α subunit of *E. coli* RNAP were found in the 44.5-kDa subunit of RNAPII (RPB3) (89), as well as in the 40-kDa (RPC40) (109) and 19-kDa (RPC19) (37) subunits that are common to RNAPI and RNAPIII (Fig. 2). A sequence similar to a region of the σ^{70} subunit of *E. coli* was noted in the fourth-largest subunit of RNAPII (RPB4) (186). Other regions with similarity to σ factors were found in some of the general RNAPII initiation factors. The TATA sequence-binding protein (TBP) contains sequences that are similar to a conserved region of σ factors which is involved in recognition of the −10 promoter region (67). The RNAP-associated protein RAP30 contains a region similar to σ^{70} (166), which in both proteins is involved in binding RNAP (102, 114). The general initiation factors TFIIB and the α chain of TFIIE also have some similarity to prokaryotic σ factors (63,

TABLE 3. Structure of *S. cerevisiae* RNAPI[a]

Size (kDa)[b]	Gene[c]	RNAP[d]	Disruption[e]	Chrom.[f]	Reference(s)[g]
190	*RPA190*		Lethal	XV	117
135	*RPA135/SRP3*		Lethal	XVI	190
49	*RPA49*		Viable		106
43	Not cloned				
40	*RPC40*	III	Lethal	XVI	109
34.5	Not cloned				
27	*RPB5*	II, III	Lethal	II	185
23	*RPB6/RPO26*	II, III	Lethal	XVI	10, 185
19	*RPC19*	III	Lethal	II	37
14.5	*RPB8*	II, III	Lethal	XV	185
12	Not cloned				
10α[h]	*RPC10*	II, III	Lethal	VIII	173
10β	*RPB10*	II, III	Lethal	XV	186

[a] Adapted from references 30, 51, and 191.
[b] Size of the subunit on sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE).
[c] Several nomenclature systems exist (see text). The genes presented in this table are all in single copy in the *S. cerevisiae* genome.
[d] Other RNAPs in which the subunit is present.
[e] Phenotype of cells carrying a chromosomal disruption of the gene.
[f] Chrom, chromosomal location.
[g] Reference(s) in which the isolation of the gene is presented.
[h] This subunit was identified recently (22).

134). These similarities suggest that eukaryotes may not have a single protein that is homologous to σ but, rather, several proteins each encoding some but not all of the functionally important regions of σ factors (78).

MUTATIONS AFFECTING ASSEMBLY OF RNAPs

Role of Zinc-Binding Regions in Assembly/Stability of RNAPs

Subunits of *S. cerevisiae* RNAPI and RNAPII that bind zinc. A zinc-blotting technique was used to identify the subunits of RNAPI and RNAPII that are capable of binding zinc (175). For both RNAPs, the two largest subunits and two common small subunits (both of 10 kDa) were shown to bind zinc. In addition, the 12-kDa subunit of RNAPI as well as the 13- and 45-kDa subunits of RNAPII bind zinc, although the latter binds only weakly.

These results are to be contrasted with earlier estimates of the zinc content of yeast RNAPI and RNAPII which were, respectively, of 1 and 2.4 zinc atoms per molecule of enzyme (12, 93). The reason for this discrepancy remains unclear.

Zinc-binding region of the largest subunit. Comparison of the amino acid sequences of conserved regions A of the largest subunit of various eukaryotic and archaebacterial RNAPs revealed the presence of a conserved zinc-binding motif of the type $CX_2CX_{6-12}CXGHXGX_{24-37}CX_2C$ (Fig. 3), which is not as easily recognizable in the β′ subunit of *E. coli*. This motif contains three pairs of residues that are capable of coordinating zinc: two cysteine pairs (CX_2C) at each end and a central mixed pair composed of one cysteine and one histidine (CXGH). Peptides corresponding to region A of *S. cerevisiae* RNAPII and RNAPIII have been expressed in *E. coli* and were shown to bind zinc in vitro in a zinc-blot assay (175, 179).

TABLE 4. Structure of *S. cerevisiae* RNAPII[a]

Size (kDa)[b]	Gene[c]	RNAP[d]	Stoic.[e]	Disruption[f]	Chrom.[g]	Reference(s)[h]
220	*RPB1/RPO21*		1.1	Lethal	IV	75, 192
150	*RPB2*		1.0	Lethal	XV	172
45	*RPB3*		2.1	Lethal	IX	89
32	*RPB4*		0.5	Conditional	X	186
27	*RPB5*	I, III	2.0	Lethal	II	185
23	*RPB6/RPO26*	I, III	0.9	Lethal	XVI	10, 185
16	*RPB7*		0.5	Viable	XII	
14.5	*RPB8*	I, III	0.8	Lethal	XV	185
13	*RPB11*			Lethal	XV	
12	*RPB9*			Conditional	VII	
10α[i]	*RPC10*	I, III		Lethal	VIII	173
10β	*RPB10*	I, III		Lethal	XV	187

[a] Adapted from references 30, 51, and 191.
[b] Size of the subunit on SDS-PAGE.
[c] Several nomenclature systems exist (see text). The genes presented in this table are all in single copy in the *S. cerevisiae* genome.
[d] Other RNAPs in which the subunit is present.
[e] Stoich., stoichiometry according to Kolodziej et al. (88).
[f] Phenotype of cells carrying a chromosomal disruption of the gene.
[g] Chrom., chromosomal location.
[h] Reference(s) in which the isolation of the gene is presented.
[i] This subunit was identified recently (22).

TABLE 5. Structure of *S. cerevisiae* RNAPIII[a]

Size (kDa)[b]	Gene[c]	RNAP[d]	Disruption[e]	Chrom.[f]	Reference(s)[g]
160	*RPO31*		Lethal	XV	128
128	*RET1*		Lethal	XV	80
82	*RPC82*		Lethal	XVI	25
53	*RPC53*		Lethal	IV	110
40	*RPC40*	I	Lethal	XVI	109
37	Not cloned				
34	*RPC34*		Lethal	XIV	169
31	*RPC31*		Lethal	XIV	126
27	*RPB5*	I, II	Lethal	II	185
25	Not cloned				
23	*RPB6/RPO26*	I, II	Lethal	XVI	10, 185
19	*RPC19*	I	Lethal	II	37
14.5	*RPB8*	I, II	Lethal	XV	185
10α[h]	*RPC10*	I, II	Lethal	VIII	173
10β	*RPB10*	I, II	Lethal	XV	187

[a] Adapted from references 30, 51, and 191.
[b] Size of the subunit on SDS-PAGE.
[c] Several nomenclature systems exist (see text). The genes presented in this table are all in single copy in the *S. cerevisiae* genome.
[d] Other RNAPs in which the subunit is present.
[e] Phenotype of cells carrying a chromosomal disruption of the gene.
[f] Chrom., chromosomal location.
[g] Reference(s) in which the isolation of the gene is presented.
[h] This subunit was identified recently (22).

The first mutations affecting conserved region A were isolated in the gene encoding the largest subunit of *S. cerevisiae* RNAPII (*RPO21*) (65). Three mutations (*rpo21-1*, *rpo21-2*, and *rpo21-3*) conferring a recessive, ts lethal phenotype were isolated that caused a slow shutoff of growth and RNAPII activity when cells were shifted to the nonpermissive temperature. Two recessive, ts mutations were also isolated in the analogous region of *S. cerevisiae* RNAPI (*rpa190-1* and *rpa190-5*) (184). Growth ceased slowly in both mutants, and both showed a decreased accumulation of RNA upon shift to the restrictive temperature. Pulse-labeling analysis performed on the *rpa190-1* mutant indicated that the synthesis of rRNA was reduced at the nonpermissive temperature whereas that of 5S RNA remained unaffected. The slow-shutoff kinetics of growth and RNA synthesis imposed by mutational alterations in the zinc-binding regions of RNAPI and RNAPII suggested that the assembly and/or stability of these enzymes was affected, rather than their catalytic function. This suggestion is supported by the finding that a strain carrying three copies of the ts allele *rpa190-1* is temperature resistant (115), probably as a result of overproduction of the mutant gene product. Immunoblot analysis showed that, in the *rpa190-1* mutant, the amount of the largest subunit decreased following a shift to the restrictive temperature, albeit to a lesser extent in a strain carrying three copies of *rpa190-1*. On the basis of these results, it was proposed that the *rpa190-1* allele encodes an unstable subunit (115). It is noteworthy that the ts phenotypes of all five mutants (*rpo21-1*, *rpo21-2*, *rpo21-3*, *rpa190-1*, and *rpa190-5*) were not remedied by the addition of zinc salts to the growth medium.

When introduced into the gene encoding the largest subunit of RNAPIII, mutations identical to *rpa190-1*, *rpa190-5*, and *rpo21-1* were lethal (179). This result suggested that RNAPIII might be more sensitive to mutations in conserved region A than is RNAPI or RNAPII (179). This suggestion is also supported by the findings that most mutations affecting invariant amino acids of the zinc-binding sequence of the

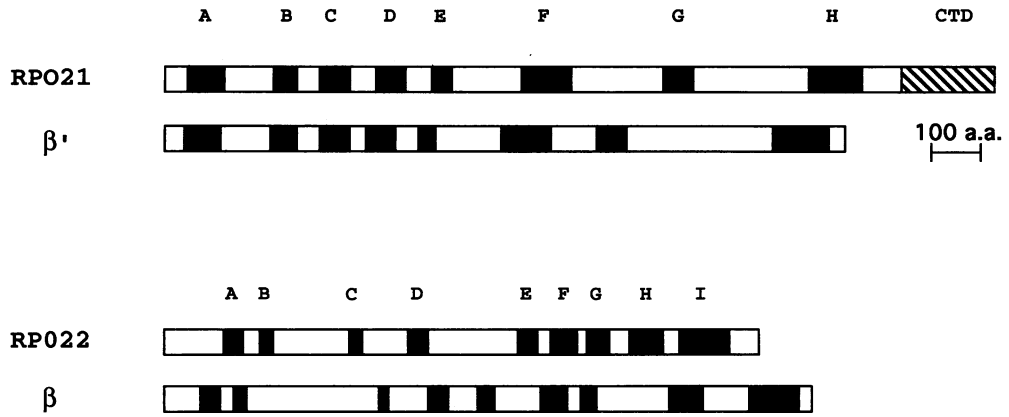

FIG. 1. Regions of sequence similarity between the two largest subunits of eukaryotic and prokaryotic RNAPs. As an example, the structures of the two largest subunits of *S. cerevisiae* RNAPII (RPO21 and RPO22) and those of *E. coli* RNAP (β' and β) are diagrammed schematically. The regions of similarity described in the text are represented as black boxes. a.a., amino acids.

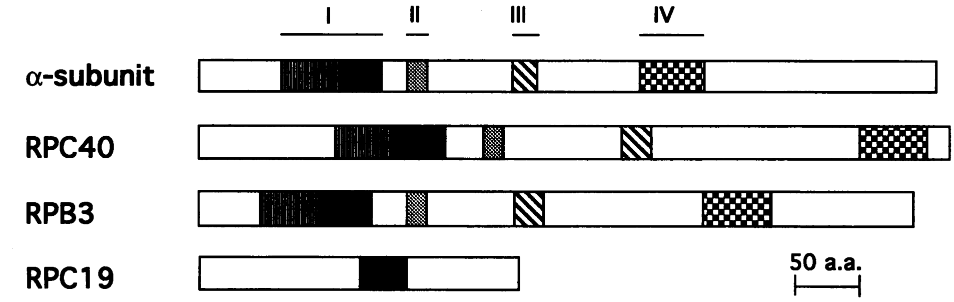

FIG. 2. Regions of sequence similarity between the α subunit of *E. coli* RNAP, the RPC40 and RPC19 subunits of *S. cerevisiae* RNAPI and RNAPIII, and the RPB3 subunit of *S. cerevisiae* RNAPII. The regions of similarity described in the text are represented as variously shaded boxes. a.a., amino acids.

largest subunit confer lethality in the context of RNAPIII (179) but only ts growth in the context of RNAPII (40). In conserved region A of *S. cerevisiae* RNAPIII, the replacement of any one of the two cysteine pairs (CX_2C) by a histidine pair (HX_2H), which potentially could also coordinate zinc, resulted in lethality. Similarly, changing the cysteine or the histidine of the central mixed pair (CXGH) for histidine and cysteine, respectively, was also lethal. Residues other than those invariably conserved were also found to be essential (179). The zinc-binding properties of three mutant proteins, each carrying a different mutationally altered pair of zinc ligands, were investigated by using a zinc blot assay (179). All three mutant proteins retained the ability to bind zinc in vitro, indicating that all six potential zinc ligands are not essential for zinc binding. On the basis of these results, it was suggested that conserved region A either binds two zinc atoms or is capable of forming two mutually exclusive zinc-binding structures (179). Two ts alleles (*rpc160-134* and *rpc160-114*) that each give rise to a single-amino-acid substitution near one of the cysteine pairs were isolated. The *rpc160-134* mutation gives rise to a mutant RNAPIII that is thermosensitive and unstable in vitro. Inactivation of the mutant polymerase in vitro was correlated with the dissociation of three subunits, of 82, 34, and 31 kDa, that are specific to the class III enzymes (179).

Zinc-binding region of the second-largest subunit. Comparison of the amino acid sequences of the second-largest subunit of various eukaryotic and archaebacterial RNAPs revealed the presence of a conserved zinc-binding motif of the type $CX_2CGX_{7-24}CX_2C$ (Fig. 4), which is not found in the β subunit of *E. coli* RNAP. A fusion protein containing this region of the second-largest subunit of *S. cerevisiae* RNAPII fused to the MalE protein of *E. coli* had the ability to bind zinc in vitro (175).

Amino acid substitutions were introduced into the zinc-binding region of the second-largest subunit of RNAPII and were assayed for their effect on zinc binding and growth of yeast cells (175). When three of the four cysteine residues thought to coordinate the zinc ion were replaced by alanine, zinc binding was greatly reduced. When introduced into the yeast genome on a low-copy-number plasmid, two of these three mutant alleles conferred a lethal phenotype while the third substitution conferred a conditional-lethal (ts) phenotype. The replacement of a cysteine by a serine resulted in a mutant protein that was altered in its ability to bind zinc in vitro. In vivo, the mutant allele gave rise to either a ts or a neutral phenotype depending on whether it was present in single or multiple copies in the yeast genome. This gene dosage effect is similar to that observed for mutations affecting the zinc finger of the largest subunit of RNAPI (see

EUKARYOTIC	RNAPI	60	NL	C	ST	C	GLDEKF	C	P	GH	Q	G	HIELPVP-CYNPLFFNQLYIYLRAS	C	LF	C	HH	107	RPA1$YEAST
		61	SV	C	AT	C	HLAERY	C	P	GH	F	G	HIVLPSP-AYHPLFFSQMYNLLRST	C	LY	C	HH	108	RPA1$SCHPO
	RNAPII	65	LK	C	QT	C	QEGMNE	C	P	GH	F	G	HIDLAKP-VFHVGFIAKIKKVCECV	C	MH	C	GK	112	RPB1$YEAST
		69	GR	C	QT	C	AGNMTE	C	P	GH	F	G	HIELAKP-VFHVGFLVKTMKVLRCV	C	FF	C	SK	116	RPB1$HUMAN
		69	GR	C	QT	C	AGNMTE	C	P	GH	F	G	HIELAKP-VFHVGFLVKTMKVLRCV	C	FF	C	SK	116	RPB1$MOUSE
		65	SR	C	QT	C	AGNMTE	C	P	GH	F	G	HIDLAKP-VFHIGFITKTIKILRCV	C	FY	C	SK	112	RPB1$DROME
		64	GR	C	MT	C	AGNLTD	C	P	GH	F	G	HLELAKP-VFHIGFLTKTLKILRCV	C	FY	C	GR	111	RPB1$CAEEL
		64	VK	C	ET	C	MANMAE	C	P	GH	F	G	YLELAKP-MYHVGFMKTVLSIMRCV	C	FN	C	SK	111	RPB1$ARATH
		66	TL	C	GT	C	NMNVKY	C	P	GH	F	G	HIELAKP-MYHYGFMNVVLNVLRCV	C	YH	C	GR	113	RPB1$PLAFD
		67	SA	C	ET	C	HRKHPE	C	P	GH	F	G	YIELAEP-VFNIGVFDLVLLVLKCV	C	KT	C	GA	114	RPB1$TRYBR
		67	SA	C	ET	C	HRKHPE	C	P	GH	F	G	YIELAEP-VFNIGVFDLVLLVLKCV	C	KT	C	GA	114	RPB2$TRYBR
	RNAPIII	65	LE	C	AT	C	HGNLAS	C	H	GH	F	G	HLKLALP-VFHIGYFKATIQILQGI	C	KN	C	SA	112	RPC1$YEAST
		72	SI	C	ET	C	GLNSIE	C	V	GH	P	G	HIDLEAP-VFHLGFFTTVLRICRTI	C	KR	C	SH	119	RPC1$TRYBR
ARCHAEBACTERIAL		58	LR	C	RS	C	GAKGGE	C	P	GH	F	G	SINLARP-VIHVGFADTIHKILSSI	C	RK	C	SR	105	RPOA$METTH
		60	LE	C	KT	C	GQRSGG	C	N	GH	F	G	HIELAAP-VIHVGFSKLIRRLLRGT	C	RE	C	AS	107	RPOA$HALHA
		56	EK	C	PV	C	GNTLAG	C	P	GH	F	G	HIELIKP-VIHIGYVKHIYDFLRST	C	WR	C	GR	103	RPOA$SULAC
VIRAL		47	AL	C	KT	C	GKTELE	C	F	GH	W	G	KVSIYKTHIVKPEFISEIIRLLNHI	C	IH	C	GL	95	RPO1$VACCV
INVARIANT RESIDUES			--	C	--	C	------	C	-	GH	-	G	------------------------	C	--	C	--		

FIG. 3. Alignment of the zinc-binding regions in conserved region A of the largest subunit of various RNA polymerases. Adapted from Werner et al. (179). The names of the sequences are those used in the SWISS-PROT data base. The subunits are from the following organisms: RPA1$YEAST, *Saccharomyces cerevisiae*; RPA1$SCHPO, *Schizosaccharomyces pombe*; RPB1$YEAST, *S. cerevisiae*; RPB1$HUMAN, *Homo sapiens*; RPB1$MOUSE, *Mus musculus*; RPB1$DROME, *D. melanogaster*; RPB1$CAEEL, *C. elegans*; RPB1$ARATH, *Arabidopsis thaliana*; RPB1$PLAFD, *Plasmodium falciparum*; RPB1$TRYBR and RPB2$TRYBR, *T. brucei*; RPC1$YEAST, *S. cerevisiae*; RPC1$ TRYBR, *T. brucei*; RPOA$$METTH, *Methanobacterium thermoautotrophicum*; RPOA$HALHA, *Halobacterium halobium*; RPOA$SU LAC, *Sulfolobus acidocaldarius*; RPO1$VACCV, vaccinia virus.

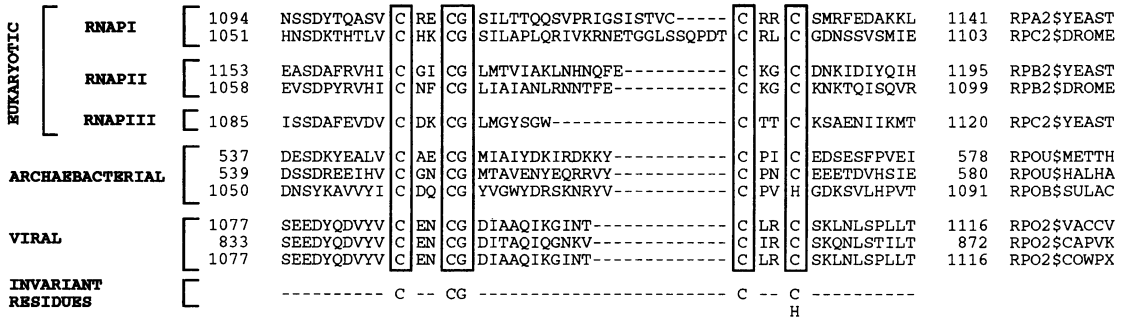

FIG. 4. Alignment of the zinc-binding regions in the second-largest subunit of various RNA polymerases. The names of the sequences are those used in the SWISS-PROT data base. The subunits are from the following organisms: RPA2$YEAST, *S. cerevisiae*; RPC2$DROME, *D. melanogaster*; RPB2$YEAST, *S. cerevisiae*; RPB2$DROME, *D. melanogaster*; RPC2$YEAST, *S. cerevisiae*; RPOU$METTH, *Methanobacterium thermoautotrophicum*; RPOU$HALHA, *Halobacterium halobium*; RPOB$SULAC, *Sulfolobus acidocaldarius*; RPO2$VACCV, vaccinia virus; RPO2$CAPVK, capripoxvirus; RPO2$COWPX, cowpox virus. As discussed previously by James et al. (80), the RPC2$DROME sequence is probably that of the second-largest subunit of RNAPI, although it has been published as the sequence of the second-largest subunit of RNAPIII.

above) and again suggests that the mutations affect the assembly of the RNAP complex rather than its catalytic function. Two mutations affecting amino acids located in the region between the two cysteine pairs of the finger retained the ability to bind zinc, although they conferred a ts or lethal phenotype. This result suggested that the ability to bind zinc is not sufficient for the function of conserved region A.

Genetic interaction between zinc-binding regions of the two largest subunits of RNAPI. Extragenic mutations that can suppress the ts phenotype conferred by two mutations (*rpa190-1* and *rpa190-5*; see above) that affect the zinc-binding region of the largest subunit of *S. cerevisiae* RNAPI have been isolated and classified into seven complementation groups, termed *SRP1* to *SRP7* (115). All suppressor mutations were dominant, and all but one (*SRP5*), suppressed the ts phenotype conferred by the *rpa190-1* and *rpa-5* mutations but not that of other *rpa190* ts mutations located outside of the zinc-binding region (115). One suppressor gene, *SRP3*, was cloned and was shown to encode the second-largest subunit of RNAPI (190). The suppressor mutation in *SRP3* gives rise to a mutant subunit containing an amino acid substitution in the zinc-binding region of the second-largest subunit. On the basis of this finding, it was proposed that the two domains interact either during assembly of the enzyme and/or within the assembled complex (190).

Extragenic suppressors of ts mutations affecting the zinc-binding region of the largest subunit of RNAPII. A search for extragenic suppressors of the ts mutation *rpo21-1* (see above) identified two related genes whose wild-type alleles have been termed *spm1*$^+$ and *spm2*$^+$ (for suppressor of polymerase mutations) (41). Comparison of the predicted amino acid sequences of both spm proteins revealed two regions of high similarity. A portion of each of these regions is also present in SIP1, a protein that interacts with the SNF1 kinase (189). Both *spm1*$^+$ and *spm2*$^+$ can be converted to dominantly acting suppressor alleles by introducing appropriate deletions in one of the regions of similarity. The suppressor alleles can also suppress other ts mutations in *RPO21* that are located outside of the zinc-binding region, as well as at least one ts mutation in an unrelated gene (*PRP4*) that is involved in pre-mRNA splicing. However, the suppressor alleles do not raise the thermotolerance of a wild-type strain. Disruption of either *spm1*$^+$, *spm2*$^+$, or both has no effect on the growth of yeast cells over a wide range of temperatures, although diploid cells homozygous for both disrupted alleles are deficient in sporulation (41). A chromosomal deletion of *spm1*$^+$ does not bring about suppression of the phenotypes imposed by *rpo21-1*. Since spm1 and spm2 proteins appear to be members of a family of proteins that binds to a protein kinase (SNF1), it was suggested that their suppressing forms could act by modulating the activity of a kinase in such a way as to increase the expression of genes whose expression is rate limiting for growth (41).

Assembly Defect Caused by ts Mutations Affecting the Two Largest Subunits of *S. cerevisiae* RNAPII

Twenty-two ts mutations were isolated by chemical mutagenesis of the gene encoding the two large subunits of *S. cerevisiae* RNAPII (130, 155, 156) and were tested for their effect on assembly of the enzyme (90). The assay used in these experiments involved the immunoprecipitation of RNAPII with an antibody directed against the third-largest subunit (RPB3). Of the 22 mutant alleles, 5 encoded polypeptides that were not assembled into an RNAPII complex when synthesized at the nonpermissive temperature. Once assembled, however, the RNAPII was stable at the nonpermissive temperature. Of those five mutations, four affect the largest subunit; two of the four affect residues that are located between conserved regions A and B, whereas the two others affect amino acids that are located between conserved regions G and H. The mutational alteration in the second-largest subunit is located between conserved regions F and G. Clearly, amino acids outside of the zinc-binding regions of the two largest subunits are also required for the structural integrity of the RNAPII complex. A mutation affecting the third-largest subunit of RNAPII (RPB3) was also shown to affect assembly of the enzyme. This result is presented below.

Genetic Interaction between the Largest Subunit of RNAPII and a Subunit Common to RNAPI, RNAPII, and RNAPIII

Nineteen mutant alleles of the gene encoding the largest subunit of *S. cerevisiae* RNAPII (*RPO21*) were created by in-frame, linker insertion mutagenesis and were classified into three groups, neutral, conditional-lethal (ts), and lethal, on the basis of their ability to support the growth of yeast cells at various temperatures (7). One of the conditional-lethal mutations, *rpo21-4*, which results in the insertion of

four amino acid residues between conserved regions F and G of the largest subunit, was chosen for further genetic characterization because it confers pleiotropic phenotypes often observed in RNAPII mutants, such as temperature sensitivity, slow growth, and inositol auxotrophy at the permissive temperature (7). The inositol auxotrophy of some RNAPII mutants, first observed by Arndt et al. (11), has now been seen for many mutations in genes encoding subunits of RNAPII. In every case in which it was examined, the Ino$^-$ phenotype could be attributed to the reduced transcription of *INO1*, which encodes the enzyme inositol-1-phosphate synthase. A gene dosage (multicopy), allele-specific suppressor of *rpo21-4* was isolated and was shown to encode the 23-kDa essential subunit (RPO26) that is common to RNAPI, RNAPII, and RNAPIII (10). On the basis of these findings and of others indicating that overexpression of the 23-kDa RPO26 protein was sufficient to bring about suppression of all the phenotypes imposed by *rpo21-4*, it was suggested that *rpo21-4* either encodes an unstable subunit that is stabilized when present in the RNAPII complex or, alternatively, encodes a mutant largest subunit which has a reduced affinity for the *RPO26* subunit (10). These findings provided some information concerning the mechanism by which mutations in genes encoding components of RNAPII give rise to pleiotropic phenotypes. It was argued that the phenotypic differences between the mutant (*rpo21-4*) and suppressed (*rpo21-4*/overexpressed *RPO26*) strains result from a difference in the amount of RNAPII being assembled in each strain and, consequently, that the ts, slow growth, and Ino$^-$ phenotypes are due to the reduced expression of a subset of genes, such as *INO1*, that are particularly sensitive to a decrease in the intracellular concentration of RNAPII (7).

A mutational analysis of *RPO26* was undertaken to identify regions of the subunit that are essential for growth and for interaction with RPO21 (10, 133). Examination of the predicted amino acid sequence of RPO26 revealed two highly charged regions: an acidic region at the amino terminus and a basic region in the middle portion of the protein (10). Results of deletion analysis indicated that the acidic region, unlike the rest of the protein, is not essential for growth (133). Several amino acid substitutions within the essential portion of this common subunit have been isolated; many of them were obtained by screening for mutations in *RPO26* that confer lethality in combination with *rpo21-4* (10, 133). Further analysis of these *RPO26* mutants will provide important information on the function of this subunit in transcription by RNAPI, RNAPII, and RNAPIII and in interaction with RPO21.

The gene encoding RPO26 is in close proximity to that encoding TFIIIA, a transcription factor required for the synthesis of 5S RNA by RNAPIII (9). The head-to-head arrangement of these two genes suggests that they might have some common regulatory elements, which could be important in coordinating the synthesis of components of the transcriptional and translational machineries.

The study presented above underscores the use of gene dosage (multicopy) suppression as a means of identifying genetic interactions between subunits of a complex enzyme. Now that most of the genes encoding the various subunits of all three *S. cerevisiae* RNAPs have been isolated, it is feasible to test directly whether a given mutation affecting a subunit of RNAP can be suppressed by overproduction of another. The usefulness of this approach was recently demonstrated by Chiannilkulchai et al. (25), who uncovered an interaction between the 82- and 31-kDa subunits of RNAPIII.

Mutations in Subunits of Eukaryotic RNAPs That Are Similar to the α Subunit of *E. coli* RNAP

Three subunits of *S. cerevisiae* RNAPs have regions of similarity with the α subunit of *E. coli* RNAP: the RPB3 subunit of RNAPII and the two subunits RPC40 and RPC19, which are common to RNAPI and RNAPIII. Four regions of sequence similarity (90) have been noted among the yeast RPB3, the human homolog of RPB3 (126), a *Tetrahymena* protein involved in conjugation (113), RPC40, and the α subunit of *E. coli* RNAP (RpoA). A portion of one of these four regions is also conserved in the RPC19 subunit (37) (Fig. 2). Genetic and biochemical evidence suggests that these regions are involved in assembly of the RNAP. In *E. coli*, analysis of truncated α subunits in vitro suggested that the amino-terminal two-thirds of the protein is required for assembly (70, 71) while the carboxy-terminal one-third is required for transcription activation by proteins such as the cyclic AMP receptor protein (70). The four regions of sequence similarity mentioned above all lie in the domain of α that is required for assembly. During assembly of RNAPII, the RPB3 subunit (probably as a dimer [88]) forms a subcomplex with RPB2, which then associates with RPO21 (90). This is reminiscent of the assembly pathway of *E. coli* RNAP (reviewed in reference 193), in which a dimer of α subunits associates with β to form an $\alpha_2\beta$ subcomplex, which then associates with β′ to form core RNAP ($\alpha_2\beta\beta'$). A ts allele of *RPB3* has been isolated (89) that carries two mutations which prevent the assembly of *S. cerevisiae* RNAPII at the nonpermissive temperature as determined by immunoprecipitation of the mutant protein following a temperature shift (90). One of the two *rpb3* mutations affects a residue in the conserved region that is also found in RPC19. It may be significant that one *rpoA* mutation (*rpoA112*) that prevents the assembly of *E. coli* RNAP also affects a residue in the same conserved region (69). Similarly, mutations affecting the assembly of RNAPI and RNAPIII have been isolated in *RPC40* (109), providing additional support for the notion that RPC40 is the α-homolog of RNAPI and RNAPIII. However, whereas there are two RPB3 or α subunits per RNAP (88, 193), there is only one RPC40 (37). For RNAPI and RNAPIII, it has been suggested that the role of one α subunit might be carried out by RPC40 whereas that of the other might be carried out by RPC19, which is also present in the polymerase complex at a stoichiometry of 1 (37). The finding that the two α subunits in *E. coli* RNAP are not functionally identical (140, 141) supports the notion that RPC40 and RPC19, together, form the equivalent of the bacterial α dimer, even though they share only one of the four α-homology regions and that both proteins are structurally different outside of this region.

MUTATIONS AFFECTING TRANSCRIPTION INITIATION

Mutations Affecting the Function of the CTD: a Domain Specific for RNAPII

The carboxy-terminal domain (CTD) of the largest subunit of eukaryotic RNAPII is composed of a heptapeptide, Tyr-Ser-Pro-Thr-Ser-Pro-Ser, tandemly repeated several times. The number of repeats varies among organisms; 26 are found in *S. cerevisiae* (3) whereas mice have 52 (29). A minimum number of repeats is required for growth (4, 14, 131, 194). In *S. cerevisiae*, mutations that shorten the CTD from 26 to 9–11 repeats confer conditional-lethal phenotypes (heat and

cold sensitivity) and inositol auxotrophy, whereas deletions that further reduce the number of repeats confer a recessive-lethal phenotype (4, 131). In yeast cells carrying only 11 of the 26 repeats, transcription of specific genes such as *INO1* and *GAL10* is reduced, whereas that of others such as *HIS4* is largely unaffected (154). This differential effect on gene expression is mediated through the upstream activator sequence elements of these genes (154). In vitro, transcription initiation at some promoters but not at others can be inhibited by antibodies directed against the CTD, by shortening the CTD (either by mutagenesis or by proteolysis), or by the addition of synthetic-peptide inhibitors (103; reviewed in references 24 and 28). In vivo, two forms of RNAPII can be distinguished on the basis of whether the CTD is highly phosphorylated or nonphosphorylated. Phosphorylation of the CTD is associated with the transition from initiation to elongation (94, 95). The nonphosphorylated form of RNAPII associates preferentially with the preinitiation complex (107), in part through a specific interaction between the CTD and the TATA-binding protein (176), and then is phosphorylated within it (107). Recent studies have demonstrated that one of the general transcription factors, known as TFIIH or BTF2 in humans, δ in rats, and factor b in yeasts, has CTD kinase activity (49, 108, 162). The gene encoding one subunit of this factor has been cloned from humans (50) and yeasts (53). Other kinases isolated from yeasts (99) and from mammalian cells ($p34^{cdc2}$ [26]) can also phosphorylate the CTD in vitro. Phosphorylation by $p34^{cdc2}$ of serine and threonine residues but not of tyrosine (195) induces a conformational change in the structure of the CTD from a compact to a more extended structure in vitro (196).

Reversion analysis of conditional-lethal mutations that retain 11 or 12 of the 26 repeats of the CTD was carried out with *S. cerevisiae* (132). Two types of *cis*-acting mutations that could suppress the ts and Ino^- phenotypes were identified: (i) mutations that lengthen the CTD, which probably arose by duplication of some of the repeats, and (ii) a mutation that gives rise to an amino acid substitution in conserved region H of the largest subunit (132).

Two suppressor genes have been identified that can suppress the phenotypes of CTD truncation mutants in *S. cerevisiae*. One of them, *SRB2*, is a dominant suppressor, suggesting that it encodes a positive regulator of CTD function. Disruption of *SRB2* is not lethal but results in phenotypes similar to those observed for CTD truncation mutants, namely high- and low-temperature sensitivity and inositol auxotrophy (87, 132). Although it is not an essential protein in vivo, SRB2 is required for basal and activated transcription in vitro. SRB2 associates with the transcription-preinitiation complex; affinity chromatography experiments with SRB2 as a ligand indicate that it can associate physically with several nuclear proteins including the TATA-binding protein (87).

The other suppressor gene, *SIN1*, was originally identified as a negative regulator of the *HO* gene (168) or as a suppressor of Ty insertion mutations (*SPT2* [144]). Mutations in *SIN1*, or its deletion, suppress the phenotypes of CTD truncation mutants. *SIN1* encodes a nonspecific DNA-binding protein with sequence similarity to HMG1, a chromatin-associated protein (91). On the basis of these genetics interactions, it has been proposed that removal of SIN1 from DNA either is mediated by the CTD or is a prerequisite for the function of the CTD (138). A role for the CTD in interaction with *trans*-activating proteins such as GAL4 has also been suggested (2).

As mentioned above, a mutation in conserved region H of the largest subunit (*rpb1-551*) was able to suppress a partial deletion of the CTD (132). This result suggests that conserved region H either participates in a function related to that performed by the CTD or can be mutated in such a way as to bypass, at least partially, the need for a full-length CTD. Since the CTD is required for transcription initiation at the promoter of certain genes, it is possible that truncation of the CTD results in a reduction in the number of initiation complexes formed at these promoters. If so, the *rpb1-551* mutation could alleviate the effect of CTD truncations indirectly by increasing the efficiency at which initiation complexes leave these promoters and enter the elongation mode (promoter clearance). The notion that region H is involved in the transition from initiation to elongation is purely speculative but is consistent with genetic data which suggest that region H is involved in a function similar to that of region I of the second-largest subunit, which, in *E. coli*, can be mutated to affect promoter clearance. The evidence that regions H and I have a similar function is based on the observation that a ts mutation affecting region H of RPO21, *rpb1-1*, which results in the rapid cessation of RNA synthesis following a temperature shift (130), can be suppressed by mutations in conserved region I of the second-largest subunit (RPB2) (111) and that, conversely, a ts mutation in conserved region I of the second-largest subunit (*rpb2-2*) can be suppressed by a mutation (*rpb1-513*) in conserved region H of the largest subunit (111).

A search for extragenic suppressors of the *rpb1-1* mutation has been conducted (112). Ten independent, extragenic suppressors were isolated and were shown to be alleles of the *KEX2* gene, which encodes a protease required for the processing of alpha-factor and killer toxin precursors (112). Suppression by mutations in *KEX2* was not specific to *rpb1-1* but was observed for many ts mutations in both *RPB1* and *RPB2* (112). The molecular mechanism by which *KEX2* influences RNAPII remains obscure.

RPB4 and RPB7: Two Dissociable Subunits of RNAPII Involved in Initiation of Transcription

Two subunits of *S. cerevisiae* RNAPII, RPB4 and RPB7, are present in the RNAPII complex at a stoichiometry of 0.5 (88), indicating that they are associated with only some of the RNAPII molecules. In vitro, these two subunits can be dissociated from the complex by treatment with urea (150) and, once dissociated, comigrate during electrophoresis under nondenaturing conditions (39). Yeast cells carrying a deletion of the *RPB4* gene are viable, although they are heat and cold sensitive as well as auxotrophic for inositol (186); RNAPII purified from this mutant strain lacks both the RPB4 and RPB7 subunits (42). A mutation affecting the largest subunit of RNAPII, *rpoB1*, has been isolated and brings about the dissociation of these two subunits from the RNAPII complex; this mutation also affects other aspects of transcription (see below). These observations suggest that the RPB4 and RPB7 subunits form a heterodimer that associates with the rest of the RNAPII complex.

The behavior of purified RNAPII lacking RPB4 and RPB7 (RNAPIIΔ4/7) was investigated in vitro (43). The rate of chain elongation and the pausing characteristics of the mutant enzyme were indistinguishable from that of the wild type. In contrast, initiation at a minimal promoter in vitro was not detected in extracts prepared from cells lacking the *RPB4* gene but could be restored by the addition of purified RPB4 and RPB7 to the extracts. Promoter-dependent initiation could also be restored by the addition of the potent

transcriptional activator GAL4-VP16, in cases when templates containing GAL4-binding sites were used. These results suggested that in the presence of transcriptional activator proteins, RPB4 and RPB7 might not be essential for initiation. This suggestion is consistent with the finding that a yeast strain lacking *RPB4* is viable. The finding that promoter-dependent initiation in extracts from a strain lacking *RPB4* could be restored by the addition of a heat-inactivated extract prepared from a strain carrying a ts mutation (*rpb1-1* [130]) affecting the largest subunit of RNAPII led to the suggestion that the RPB4 and RPB7 subunits can shuttle between RNAPII molecules (43).

A mutation, *rpoB1*, was isolated (180) that affects the largest subunit of *S. cerevisiae* RNAPII and brings about the dissociation of the RPB4-RPB7 subcomplex during purification (150). Comparison of the enzymatic properties of the *rpoB1* enzyme with wild-type RNAPII or wild-type RNAPIIΔ4/7 indicated that the mutation also affected initiation and elongation as measured by the amount of incorporation of the initiation and elongation nucleotides, respectively, during promoter-independent transcription in vitro (150). The ability of the mutant enzyme to bind denatured DNA was also investigated and was found to be similar to that of the wild-type enzyme (150).

Mutations That Stimulate Transcription Initiation in the Absence of Transcriptional Activator Proteins

Mutations affecting the two largest subunits of *S. cerevisiae* RNAPII were isolated by Arndt et al. (11) in a genetic selection for suppressor mutations that can restore transcription of *HIS4* in a strain in which three genes, *BAS1*, *BAS2*, and *GCN4*, have been deleted. All three genes encode factors that act at the *HIS4* promoter to activate transcription. A yeast strain carrying this triple deletion has a His^- phenotype because *HIS4* is poorly transcribed. The isolation of suppressor mutations that restored a His^+ phenotype identified four genes, termed *SIT1* through *SIT4* (for suppressors of initiation of transcription). *SIT1* and *SIT2* were shown to encode the largest and second-largest subunits, respectively, of RNAPII. Nucleotide sequence analysis of several *sit1* alleles revealed that some encode mutant largest subunits with amino acid substitutions in conserved region F (6). *SIT3* is allelic to *GCR1*, a gene that is required for high-level expression of genes encoding many glycolytic enzymes. *SIT4* encodes a protein with sequence similarity to the catalytic subunit of protein phosphatases.

In *sit1* through *sit4* strains, transcription of *HIS4* is initiated at the normal start sites and retains a requirement for the TATA sequence (with the exception of *sit3*, which does not require it), as well as the binding of RAP1, a protein that binds to the *HIS4* promoter but does not efficiently stimulate its transcription in the absence of BAS1, BAS2, and GCN4 (11, 38). Mutations in *sit1* through *sit4* affect the expression of several unrelated genes. Genes such as *FUS1* and *FUS2*, which are normally induced by mating pheromone, are expressed at levels higher than basal in certain *sit* strains in the absence of pheromone. Similarly, low levels of expression of *GAL1* are detected in *sit1* through *sit4* strains grown in the presence of glucose, which normally represses transcription of the *GAL* genes. *sit1* and *sit2* strains grown in the absence of inositol fail to derepress *INO1*. The altered pattern of gene expression detected in *sit1* through *sit4* strains most probably underlies the numerous phenotypes of these mutants such as temperature sensitivity, low growth rate at the permissive temperature, inositol auxotrophy, and poor growth on nonfermentable carbon sources. On the basis of these observations and the finding that pairwise combinations of *sit1*, *sit2*, and *sit4* are lethal, Arndt et al. (11) proposed that *SIT4* encodes a protein phosphatase that dephosphorylates the largest subunit of RNAPII, perhaps on the CTD. In *sit4* strains, more of the RNAPII molecules would be in the phosphorylated state, which would result in aberrant gene expression. Subsequently, it was found that SIT4 is required for cell cycle progression from late G_1 into S phase and that it is located in the cytoplasm (171). The relationship between these findings and the role of *SIT4* in transcription remains unclear.

Mutations Affecting Start Site Selection by RNAPII

In *S. cerevisiae*, mutations conferring either a His^- or a Lys^- phenotype have been isolated that are caused by the insertion of a Ty retroposon (or simply of its long terminal repeat, called solo δ-element) in the promoter region of *HIS4* or *LYS2*. One such mutation, *his4-912*δ, is an insertion of the Ty δ-element between the *HIS4* upstream activator sequence and TATA sequence that creates a tandem promoter. Previous genetic analysis suggested that the His^- phenotype caused by the *his4-912*δ mutation is the result of a competition between the two promoters: instead of initiating at the normal *HIS4* initiation site, transcription is initiated at a site in the δ-element to produce a longer mRNA that is not functional, probably because of the presence of translation initiation and termination codons upstream of the *HIS4* open reading frame (66). Both *cis*- and *trans*-acting mutations that restore a His^+ phenotype by stimulating transcription initiation at the *HIS4* start site have been isolated (48, 66, 181–183). Some of the *trans*-acting suppressor genes, known as *SPT* (for suppressor of Ty), encode various products that are involved directly or indirectly in transcription, such as the general initiation factor TFIID (*SPT15* [44]), the transcription factor GAL11 (*SPT13* [47, 48]), or histones (*SPT11-SPT12* [27]). The situation created by the δ-insertion mutation *lys2-128*δ at the *LYS2* locus is analogous to that observed at the *his4-912*δ allele in that two transcription start sites are found, one of which produces a nonfunctional mRNA. Mutations in the genes encoding the two largest subunit of *S. cerevisiae* RNAPII that can cause reversion of the His^- and Lys^- phenotype of a strain carrying the *his4-912*δ and *lys2-128*δ mutations were isolated (64). Two recessive mutations in *RPB1* affecting residues in conserved region G, as well as four semidominant mutations in *RPB2* affecting residues located between conserved regions C and D, were found that increase the synthesis of the functional mRNAs without affecting the levels of the nonfunctional mRNAs. Other than reversion of the histidine and lysine auxotrophies, the *rpb1* and *rpb2* mutations had no discernible effect on the growth of yeast cells over a wide range of temperature or on their mating and sporulation efficiencies. In contrast, yeast cells carrying both *rpb1* mutations in the same gene were inviable. At *HIS4*, the *rpb1* mutants still required GCN4, BAS1, and BAS2 for transcription and had a small but detectable effect on the pattern of start sites; the level of a minor transcript was elevated in the *rpb1* mutant strains relative to the wild type. In contrast, the *rpb1* mutations had no effect on the pattern of start sites of the *CYC1* and *CYH2* genes. These results suggested that RNAPII itself plays a role in the selection of the transcription start sites.

The finding that the *rpb1* mutations affect residues in conserved region G is intriguing in light of recent cross-

linking experiments, which suggest that the analogous region of the *E. coli* β′ subunit is part of the catalytic center of the enzyme. In the *E. coli* experiments, the 3′ end of the nascent RNA was cross-linked to RNAP and a contact site was mapped to an 89-amino-acid region of the β′ subunit which includes a portion of conserved region G (18). It has been suggested that the *rpb1* mutations might bring about the mutant phenotype by altering the conformation of the active site of RNAPII (64).

Initiation Nucleotide-Binding Site

Affinity-labeling experiments with photoreactive nucleoside triphosphate analogs have been performed in an attempt to identify regions of RNAP that are involved in binding the initiation nucleotide. For *E. coli* RNAP, *Sulfolobus acidocaldarius* RNAP, and *S. cerevisiae* RNAPII, these experiments identified several candidate lysine residues in conserved region H of the second-largest subunit (between amino acids 1036 and 1066 of β), one of which is invariably conserved (54–56, 142). Replacement of this conserved lysine by arginine in *S. cerevisiae* RNAPII (amino acid 979) resulted in a recessive lethal phenotype (174), whereas in *E. coli* RNAP (position 1065 in the β subunit) it gave rise to a mutant enzyme that inhibited cell growth (85) and prevented the cross-linking of the β subunit by certain nucleotide analogs (129). The dominant-negative phenotype was easily explained by the finding that, in vitro, the mutant RNAP could bind to promoter regions as well as wild-type RNAP, could catalyze the formation of the first phosphodiester bond, but failed to engage in the elongation process. This behavior of the mutant enzyme was similar to that of the wild-type enzyme inhibited by the antibiotic rifampin, suggesting that conserved regions H and D (the rif region) of the β subunit perform a common function.

The use of longer nucleotide analogs allowed the labeling of a second region of *E. coli* RNAP and *S. cerevisiae* RNAPII, which is located at the carboxy terminus of the second-largest subunit. For *E. coli* RNAP, several candidate lysine residues in conserved region I (between amino acids 1234 and 1242), as well as histidine 1237, were identified as possible labeling sites (54, 55). The change of His-1237 for alanine in the β subunit resulted in a recessive-lethal phenotype; in vitro the mutant RNAP displayed a reduced promoter clearance ability (129). For *S. cerevisiae* RNAPII, lysine 1102 of conserved region I was identified as the affinity-labeled residue (174). Replacement of lysine 1102 by arginine did not affect the growth of yeast cells (174).

Dominant-Negative Mutation Affecting Promoter Clearance and Rate of Elongation

In *E. coli*, a dominant-lethal mutation in the *rpoB* gene (encoding the β subunit of RNAP) was isolated that gives rise to the substitution of lysine for the conserved glutamic acid 813 in conserved region F (97, 98). In vitro, the purified enzyme displays a modified pattern of abortive transcripts, a decreased frequency of promoter clearance, and a decreased elongation rate. The defect in promoter clearance and elongation rate could be compensated for by raising the nucleoside triphosphate (NTP) concentration, suggesting the possibility that the mutation affects the ability of the enzyme to bind the elongation substrate. However, this suggestion was not supported by the pattern and abundance of abortive transcripts generated by the mutant and wild-type enzymes, which were indistinguishable at various nucleotide concentrations. To account for these findings, the authors suggested that the mutation affects primarily the stability of the nascent RNA in the active site of the polymerase. In the abortive-initiation process, a defect in binding the nascent RNA could lead to the loss of the short oligomer already synthesized. During the elongation process, a defect in positioning the nascent RNA to form part of the incoming nucleotide-binding site could result in an increased K_m. These results suggested that a common mechanism, which is affected by the E813K mutation, allows the RNAP to clear the promoter and to be released from pausing sites (97). In a subsequent study, the behaviors of the mutant and the wild-type enzyme were studied at the *lacUV5* promoter in the absence or presence of the *lac* repressor in vitro (96). It was shown that the effect of *lac* repressor is to modify the catalytic activity of RNAP such that the enzyme displays an apparently higher K_m for each incoming nucleotide. When present in a joint complex with *lac* repressor, RNAP remains capable of synthesizing short abortive transcripts in a reaction that is very sensitive to a decrease in the substrate concentration but is incapable of transcribing through a site with a high K_m. In the absence of the repressor, RNAP is able to traverse this pausing site and clear the promoter. This inability of RNAP to traverse a site with a high K_m can also be observed in the absence of the repressor, either if the nucleotide substrate concentration is low or if the RNAP carries the E813K mutation. A similar site with a high K_m was found in the *bla* and *T7A1* promoters, suggesting that the existence of such sites might be widespread (96, 97).

MUTATIONS AFFECTING TRANSCRIPTION ELONGATION, PAUSING, AND TERMINATION

Mutations in *E. coli rpoB* That Alter Transcription Elongation, Pausing, and Termination

Random, chemical mutagenesis of the cloned *rpoB* gene of *E. coli* was used to create mutations that increase or decrease termination by RNAP. A large collection of mutations was isolated that increase termination at the *trp* attenuator or decrease termination at a rho-independent terminator located upstream of a *cat* gene. Most of the mutations were found to affect residues in three distinct regions of the β subunit (92): conserved regions D (amino acids 500 to 575 of β), F (740 to 840), and I (1225 to 1342). Many of the altered residues are conserved in prokaryotic and eukaryotic enzymes. The mutations conferring the strongest effect on transcription termination and pausing affect residues from amino acids 500 to 575. This is the same region which encompasses most of the mutational alterations leading to resistance to the transcription inhibitor, rifampin. This result was not unexpected since most Rif^r mutants display an altered termination and antitermination phenotype in vivo. Landick et al. (92) argued that most of their mutations altered termination and pausing by stabilizing or destabilizing the ternary complex in its elongation configuration relative to its paused or terminating configurations. This could be accomplished if the mutations affect contacts between the β subunit and either the DNA template, the nascent RNA transcript, or the RNA-DNA heteroduplex (92). An alternative model based on kinetic arguments was also proposed: the mutations could affect pausing and termination by altering the elongation rate of the RNAP. The latter view is supported by the study of the *rpoB8* mutant by Jin and Gross (82) presented below.

Mutation Affecting Nucleotide Binding, Elongation Rate, and Termination

The *rpoB8* mutation in *E. coli* confers resistance to rifampin and increased termination efficiency at rho-dependent and rho-independent terminators in vivo. In vitro, the purified enzyme was more prone to pausing and had a slower elongation rate than the wild-type enzyme (82). These two phenotypes could be explained by the finding that the mutant enzyme displayed an increased K_m for purine nucleotides during elongation; this is in agreement with the finding that the pausing defect and the reduction of elongation could be compensated for by raising the nucleotide concentrations in vitro. In contrast, the K_m for ATP during initiation was found to be similar to that of the wild-type enzyme. These results provided support for the notion that the rate of elongation and the efficiency of termination at intrinsic terminators are coupled kinetically; it also suggested that the residues affected by the Rif^r mutations formed part of the binding site for the elongating substrate nucleotide. Kinetic coupling between the rate of transcription by RNAP and the rate of action of rho has been shown to affect the efficiency of termination at rho-dependent terminators (81).

Relationship between Elongation Rate and Transcriptional Accuracy

A mutation in *rpoB* that increases the elongation rate of RNAP has been isolated in a selection for mutations that decrease transcriptional accuracy (17). The level of transcription error by *E. coli* RNAP, which has been estimated to be 1×10^{-5} to 10×10^{-5} both in vivo and in vitro (reviewed in reference 104), suggests that the fidelity of RNA synthesis is actively maintained by a process of error avoidance and/or error correction (104). The *rpoB* mutation described by Blank et al. (17) was isolated by screening a collection of Rif^r mutants for those that increase the background leakiness of a strongly polar nonsense mutation located near the beginning of the *lacZ* gene. The rationale for the mutant isolation (discussed in reference 17) is based on the suggestion that the leakiness of this polar mutation is due mainly to transcriptional errors. One *rpoB* mutant, named *ack-1* (for "ackuracy"), was isolated and the mutation was mapped to conserved region D between residues 565 and 576 of the rif region (17, 105). In vitro, RNAP purified from *ack-1* displays a twofold increase in the level of misincorporation (17). The ability of wild-type RNAP to hydrolyze incorrect NTPs into their corresponding nucleoside diphosphates (NDPs) during elongation is missing in the mutant *ack-1* RNAP, suggesting that hydrolysis of noncognate nucleotides is important for transcriptional accuracy (105). It has been suggested that the combination of error avoidance by using hydrolysis of noncognate rNTPs with error correction, perhaps by using the pyrophosphorolysis activity of RNAP, could ensure a high fidelity of RNA synthesis (104). The finding that transcription of the *lacZ* gene in vivo by *ack-1* RNAP is slightly faster (about 15%) than by wild-type RNAP (105) suggests that discrimination of complementary from noncomplementary rNTPs contributes to the overall rate of chain elongation.

α-Amanitin Resistance and Transcription Elongation

Mutation conferring α-amanitin resistance affects elongation by RNAPII in vitro. An α-amanitin-resistant *D. melanogaster* mutant (*C4*) was shown to carry a mutation in an X-linked gene (*RpII215*) that gives rise to an altered RNAPII (58) with reduced affinity for α-amanitin (31). Subsequently, the gene conferring α-amanitin resistance was cloned by P-element tagging (159) and was shown to encode the largest subunit of RNAPII (57). By using the "tailed-template" assay developed by Kadesch and Chamberlin (84), the mutant C4 RNAPII was shown to elongate more slowly than its wild-type counterpart, demonstrating a role for the largest subunit in transcript elongation (32). The finding that the C4 enzyme displayed an increased K_m for UTP whereas that for GTP was near the wild-type value provided an explanation for the decreased elongation rate (the K_m values for ATP and CTP were not measured) (32). The increased K_m for UTP does not necessarily indicate that the C4 mutation affects a region of RNAPII involved in substrate binding, since a defect in the ability of the enzyme to translocate could lead to an increase in the measured K_m without affecting substrate binding (86). The proposal that the C4 enzyme fails to translocate efficiently is attractive in light of the finding that α-amanitin inhibits the translocation step of the elongation process (36, 153).

Defining the α-amanitin-binding site. To date, only in the mouse has a mutation conferring α-amanitin resistance been precisely localized within the gene encoding the largest subunit (13). The gene encoding the largest subunit of mouse RNAPII (*RpII215* [1]) was isolated from an α-amanitin-resistant cell line and was used in transfection experiments to map the resistance mutation: a single-nucleotide change that results in the substitution of an asparagine residue for an aspartic acid in conserved region F of the largest subunit. This asparagine residue is conserved in the largest subunit of mammalian RNAPII but not in that of RNAPI, RNAPIII, and *E. coli* RNAP, which is consistent with its association with α-amanitin sensitivity. Furthermore, a serine residue is found at the corresponding position of *S. cerevisiae* RNAPII, which agrees with the finding that the yeast enzyme is 500-fold less sensitive than its mammalian counterpart. The α-amanitin mutation also confers the resistance phenotype when introduced into mouse embryonic stem cells by gene targeting (167). These cells should allow the investigation of the effect of this mutation on mouse development.

Isolation of a *C. elegans* RNAPII that is superresistant to α-amanitin. Sanford et al. isolated the first *C. elegans* mutant resistant to α-amanitin and showed that RNAPII purified from it was 150 times more resistant to the toxin than the wild-type enzyme was (152). This mutation defined the *ama-1* gene. Starting with a mutant carrying a resistant allele of *ama-1*, Rogalski et al. (146) isolated a second mutation within it that further increased the level of α-amanitin resistance. RNAPII present in this double mutant is 20,000-fold more resistant to α-amanitin than the wild-type enzyme is, as measured by inhibition of RNA synthesis in isolated nuclei. Previously isolated α-amanitin-resistant RNAPII in *C. elegans*, *D. melanogaster*, or cultured mammalian cells were typically 100- to 200-fold more resistant than the wild-type enzyme was. Fine genetic mapping indicated that the two *C. elegans* mutations are less than 200 bp apart. The "superresistant" polymerase was unstable during purification, and the double mutant displayed a ts sterile phenotype. These results suggest that it may not be possible mutationally to inactivate the α-amanitin-binding site on RNAPII without causing concomitant loss of enzyme activity. However, one should keep in mind the existence of RNAPII enzymes that are naturally resistant to α-amanitin, such as that of *Aspergillus nidulans* (170).

Genetic Interaction between Transcription Elongation Factor TFIIS and the Largest Subunit of RNAPII

TFIIS is an elongation factor that binds to RNAPII and facilitates its passage through pausing sites. As a first step toward identifying regions of RNAPII that are involved in transcript elongation and interaction with TFIIS, mutations in the gene encoding the largest subunit of *S. cerevisiae* RNAPII were isolated that confer increased growth inhibition by the uracil analog, 6-azauracil (6AU) (7). The 6AU-sensitive phenotype is associated with mutants lacking the nonessential gene *PPR2* (45, 68), which encodes the *S. cerevisiae* transcription elongation factor TFIIS. Therefore it was anticipated that mutations in *RPO21* conferring 6AU sensitivity could affect the elongation properties of RNAPII. Five linker insertion mutations and two point mutations isolated by random chemical mutagenesis of a cloned copy of *RPO21* were found to confer sensitivity to 6AU. All seven mutational alterations are clustered in a single, essential region of the largest subunit (between conserved regions G and H), which is conserved in RNAPII but which is not found in the largest subunit of RNAPI and III or that of *E. coli*. The suggestion that the 6AU-sensitive *rpo21* mutants are defective in the process of elongation was supported by the finding that the 6AU sensitivity is alleviated by a high dosage of the *PPR2* gene, presumably as a result of overexpression of TFIIS. On the basis of these results, it was proposed that, in the *rpo21* mutants, the formation of the RNAPII-TFIIS complex is rate limiting for the passage of the mutant enzyme through pausing sites either because the mutant RNAPII is more prone to pausing or because it has a reduced affinity for TFIIS (8).

Mutations Affecting Transcription Termination by Eukaryotic RNAPs

In *S. cerevisiae*, mutations that decrease termination by RNAPIII were sought by using a selection based on an allele of the *SUP4* tRNA suppressor gene that carries a weak terminator signal in its intervening sequence as a result of a mutational event that created a tract of T residues (the major component of the RNAPIII transcription termination signal). It was expected that nonlethal mutations that decreased termination at this weak terminator would increase the synthesis of the suppressor tRNA, which in turn could be detected by an increase in phenotypic suppression of ochre-suppressible auxotrophic markers. One recessive mutation, *ret1-1* (for reduced efficiency of termination), was isolated that decreased termination at weak terminators in vivo as well as in vitro in S-100 extracts (79). *ret1-1* has no effect on the growth rate and mating and sporulation efficiency of yeast cells. The wild-type *RET1* gene was cloned and shown to encode the second-largest subunit of RNAPIII. The *ret1-1* allele was also isolated, and the mutation, a threonine-to-lysine substitution at position 311, was mapped between conserved region B and C (80). It may be significant that the *ret1-1* mutation lies in a region of the second-largest subunit that has weak sequence similarity with bacterial RNases (163). Further biochemical experiments should prove useful in determining the molecular defect underlying the termination phenotype of the *ret1-1* RNA polymerase III.

In *D. melanogaster*, a ts recessive-lethal mutation in *RpII215*, *WJK1*, was isolated (122) that is thought to give rise to a mutant RNAPII which is more prone to premature termination at the restrictive temperature. This suggestion was based on the results of experiments in which transcription of a reporter gene, expressed from the *Hsp82* promoter and carrying an insertion of a polyadenylation signal in its intron, was examined in cells homozygous for the *WJK1* mutation (119). Because of the polyadenylation signal inserted in its intron, the reporter gene gives rise to two transcripts of different sizes: a truncated transcript and a full-length processed transcript. As a control, accumulation of the full-length processed transcript originating from the endogenous *Hsp82* gene was also examined. Following heat shock, accumulation of the shorter transcript but not of the two full-length ones was observed in cells homozygous for *WJK1*. This is in contrast to wild-type cells, which accumulate all three transcripts under the same conditions. An allele-specific suppressor of *WJK2* has been isolated that suppresses both the ts lethality and "premature-termination" phenotypes. The suppressor mutation lies in the gene encoding the second-largest subunit of RNAPII (119). The isolation of extragenic suppressor mutations of *WJK2* also identified two other loci that are likely to encode additional components of the transcription apparatus (119).

PLEIOTROPIC PHENOTYPES OF RNAP MUTANTS

Mutations Affecting Development

Development of *D. melanogaster*. The role of RNAPII in the development of *D. melanogaster* was first realized when mutations in *RpII215* (encoding the largest subunit) were found to be allelic with the lethal-mutable locus *L5* (31, 59, 100). A recessive-lethal allele of *RpII215*, *Ubl*, was isolated that conferred a dominant phenotype similar to that imposed by mutations in *Ultrabithorax* (*Ubx*), which encodes a transcriptional regulatory protein (123). In the developing fly, *Ubx* causes a partial transformation of a part of the halter structure, the capitellum, into a wing. This transformation is accentuated in *Ubl* mutant flies also carrying a mutant allele of *Ubx*. This enhancement of the *Ubx* phenotype was also observed for other mutations either in *RpII215* (including the well-characterized mutation *C4*) (122) or in *RpII140*, the gene encoding the second-largest subunit of RNAPII (125). However, in contrast to mutations affecting the largest subunit, mutations in *RpII140* do not lead to the transformation of the capitellum into wing in the absence of *Ubx* mutations (125). The study of mosaic flies, in which cells carrying either *Ubl* and the wild-type allele or only the *Ubl* allele could be distinguished, revealed that the mutant phenotype conferred by *Ubl* and its enhancement by *Ubx* both require the expression of the wild-type *RpII215* (124). To account for the antagonistic interaction between *Ubl* and *RpII215*, Mortin et al. (124) suggested that cells carrying both alleles might be incapable of appropriate regulation of the amount of RNAPII, whereas cells carrying only *Ubl* might be able to do so correctly. Alternatively, the two forms of RNAPII (wild type and Ubl) present in the same cells might interfere with one another at some step during transcription (124). Similar antagonistic interactions have been observed for other combinations of mutant and wild-type alleles of *RpII215* (120, 122, 124, 178) or of *RpII140* (125). In addition to enhancing the mutant phenotype of *Ubx*, certain mutations in *RpII215*, including *Ubl* and *C4*, can enhance the phenotype of other mutations such as those in the *Delta* (*Dl*) locus (122). Unlike enhancement of the Ubx phenotype, enhancement of Dl does not appear to result from an antagonism between *RpII215* alleles (122).

The developmental effects of a recessive ts allele,

RpII215^{ts}, have also been well characterized (120, 121). In vitro, the *RpII215*^{ts} allele gave rise to an RNAPII with altered activity (31). Adult flies carrying this mutation are sterile at the nonpermissive temperature (120). Zygotes homozygous or hemizygous for the mutation developed until the late embryonic or first larval instar stage at the restrictive temperature. The lethal effect of this mutation was correlated with a ts period between gastrulation and pupation. Since zygotes lacking the *RpII215* gene develop until the late embryo stage, the early defects conferred by the *RpII215*^{ts} mutation are most probably due to a reduced and/or abnormal transcription brought about by a reduction in the loading of maternally encoded RNAPII or by the loading of thermosensitive RNAPII (120, 121). Intragenic suppressor mutations of *RpII215*^{ts} have been isolated and should prove useful in identifying interacting regions of the largest subunit of RNAPII (119).

A recessive-lethal mutation, *wimp*, affecting the second-largest subunit of RNAPII has been isolated; it confers a dominant maternal effect (136). *wimp* causes a twofold reduction in the level of expression of certain maternal and zygotic genes. Phenotypically, flies heterozygous for a recessive mutation in one of the interacting genes display an intermediary mutant phenotype in the presence of *wimp*. In the absence of other interacting mutations, flies heterozygous for the *wimp* mutation develop normally despite a reduction in transcription of certain genes. On the basis of the latter result, Parkhurst and Ish-Horowicz (136) suggested that a reduction in the transcription of certain genes might be tolerated if accompanied by a compensatory reduction in the transcription of others.

Development of *C. elegans*. The availability of dominant mutations conferring resistance to α-amanitin in *C. elegans* allowed the isolation of recessive lethal mutations in *ama-1* (encoding the largest subunit of RNAPII) that were identified by scoring for loss of the resistance phenotype (145, 147). Many of these lethal mutations are probably null, since they confer a phenotype similar to that of a mutant carrying a deletion of *ama-1* (145, 147); these mutants were able to complete embryogenesis, presumably by using maternally encoded RNAPII, but arrested development as L1 larvae. One mutation did not allow completion of embryogenesis, suggesting that the mutant allele encodes a gene product that can interfere with wild-type RNAPII function (145, 147). Some of the recessive-lethal mutations allowed development to proceed to stages later than L1, whereas others allow its completion but confer sterility (145, 147). For some mutants the severity of the phenotype was changed by varying the temperature (145, 147). Fine-structure genetic analysis allowed the mapping of 16 of the recessive-lethal mutations relative to a dominant α-amanitin resistance mutation and, for some mutations, relative to each other (20). Intragenic suppressors of some of the recessive lethal mutations have been isolated (145). The molecular cloning and nucleotide sequence determination of *ama-1* (16) will facilitate the precise localization of the recessive lethal and suppressor mutations and should provide important information on the relationship between the structure and function of the largest subunit of RNAPII.

Effect of Mutations in Cultured Cell Lines

Isolation of α-amanitin-resistant cell lines. The first α-amanitin-resistant cell line was isolated from CHO cells and was shown to contain an altered RNAPII (23). Subsequently, other α-amanitin-resistant lines carrying an altered RNAPII activity were isolated from CHO cells (74), rat myoblast L6 cells (164), Syrian hamster BHK-T6 cells (5), mouse myeloma cells (188), and human fibroblasts (19). In most resistant cell lines, the resistant and sensitive enzymes were both synthesized, indicating that the resistance mutation was codominantly expressed. In contrast, some CHO cells lines were found to be functionally hemizygous for the resistance mutation, a property that was later exploited to isolate recessive ts α-amanitin-resistant mutations affecting the largest subunit as well as revertants of two of these ts mutations (72).

Regulation of RNAPII biosynthesis. The availability of cell lines carrying both Ama^s and Ama^r alleles allowed the study of the regulation of RNAPII levels. It was shown that growth of these cells in the presence of α-amanitin resulted in the loss of the wild-type RNAPII activity concomitant with an increase of the resistant activity (61, 165). In Ama^s/Ama^s/Ama^r hybrid cells, immunoprecipitation with an antiserum directed against RNAPII showed that the polypeptide compositions of resistant and sensitive RNAPIIs were identical and that the increase of the resistant activity relative to the wild type was associated with the coordinate increase in the rate of synthesis of at least three subunits of RNAPII, the largest subunit (214 kDa), a common subunit (25 kDa), and a smaller one (20.5 kDa), whereas the rate of synthesis of others, like that of the second largest subunit, was unchanged (62). This increase in the rate of synthesis of certain subunits was accompanied by the selective degradation of the sensitive enzyme as determined by the increased rate of degradation of the largest subunit and, to a lesser extent, of the second largest (62).

Effect of α-amanitin resistance mutations on cellular differentiation. The effect of mutations conferring α-amanitin resistance on the ability of L6 rat myoblast to undergo myogenic differentiation was investigated by Crerar et al. (33). It was found that α-amanitin-resistant mutants were two to three times less likely to undergo myogenic differentiation as measured by their ability to form myotubes and synthesize proteins characteristic of muscle tissue. For certain α-amanitin-resistant mutants, the defective myogenic differentiation phenotype was observed only when cells were cultured in the presence of α-amanitin. This conditional phenotype was probably due to a regulatory phenomenon similar to the one described by Guialis et al. (62), which leads to the loss of the wild-type RNAPII when cells are grown in the presence of α-amanitin.

Effect of RNAPII and RNAPIII mutations on cell cycle progression. A Syrian hamster BHK21 ts cell line (TsAF8) that arrested in mid-G_1 of the cell cycle at the nonpermissive temperature (21) was isolated by Meiss and Basilico (116). In this mutant line, RNAPII activity is deficient as measured in isolated nuclei (148) and in soluble extracts (149), suggesting that the defect might be caused by a mutation affecting a subunit of RNAPII. This suggestion was supported by the results of gene transfer experiments in which the ts phenotype of TsAF8, or of an α-amanitin-resistant derivative of TsAF8, was reverted by the introduction of DNA from an α-amanitin-resistant or sensitive TS$^+$ line; in every case, reversion of the Ts phenotype of TsAF8 was accompanied by the acquisition of the α-amanitin-sensitive or -resistant phenotype of the donor cell line (76). In these revertant lines, DNA from the donor could be detected by using probes from the *D. melanogaster RpII215* locus that contained part of the structural gene encoding the largest subunit of RNAPII (73).

In *S. cerevisiae*, six conditional-lethal mutations in the *RPC53* gene encoding a subunit of RNAPIII were isolated

that preferentially arrested in the G_1 phase of the cell cycle, as large unbudded cells, when shifted to nonpermissive conditions (110). The RPC53 subunit is significantly similar to the human protein BN51 (110). BN51 also appears to be essential for progression through the cell cycle since its cDNA was isolated by its ability to complement the ts defect of a BHK mutant cell line that arrest in the G_1 phase of the cell cycle at the restrictive temperature (77). These findings raised the possibility that BN51 is the mammalian homolog of the RPC53 subunit of RNAPIII.

Effect of RNAP Levels on Gene Expression

RNAPIII. In *S. cerevisiae*, four ts mutations in the gene encoding the largest subunit of RNAPIII (*RPO31*) have been isolated that cause a reduced growth rate at the permissive temperature (60). For two of the mutants, the levels of synthesis of tRNA and 5S RNA were measured at the permissive temperature or following a shift to the nonpermissive temperature. Although a reduction in the accumulation of tRNAs was observed at the permissive temperature and to a greater extent following a shift to the nonpermissive temperature, very little effect on the level of accumulation of 5S RNA was found (60). Since this study, a similar effect on 5S and tRNA synthesis was observed in many other RNAPIII mutants carrying mutant alleles of either *RPC82* (25), *RPC53* (110), *RPC34* (169), or *RPC31* (126). In the latter case, a null allele of *RPC31* was constructed by replacing a tyrosine codon by the nonsense codon UAA (126). A strain carrying this null allele and a tyrosine-inserting tRNA suppressor produces a wild-type RPC31 protein, although in smaller amounts because of the inefficiency of nonsense suppression. This mutant displays a twofold reduction in growth rate and in the synthesis of tRNAs. In contrast, very little effect was detected on the synthesis of 5S RNA. These results strongly suggest that a decrease in the intracellular concentration of RNAPIII is sufficient to bring about a differential effect on gene expression. It has been suggested either that the mutant RNAPIII could have a lower affinity for the initiation complexes assembled at the tRNA genes than for those assembled at the 5S RNA gene or that the mutant enzyme is more stable in the nucleolus (where transcription of 5S occurs) than in the nucleoplasm (where tRNAs are synthesized) (25, 60, 110, 169).

The finding that the synthesis of 5S RNA is particularly sensitive to mutations affecting RNAPIII is reminiscent of the observation that transcription of the *INO1* gene is reduced in many RNAPII mutants, presumably as a consequence of a reduction of the intracellular concentration of RNAPII (7).

RNAPII. Experiments similar to those described above, in which the amount of one subunit is made limiting for the assembly of RNAP, were performed with *D. melanogaster*. *cis*-acting mutations affecting the level of expression of the gene (*RpII215*) encoding the largest subunit of RNAPII have been isolated in a search for revertants of a lethal allele of *RpII215* that had been created by the insertion of a transposable P element in the 5′ noncoding region of the transcribed sequence (158, 159, 177). Revertants were isolated in which the activity of *RpII215* had been restored either totally or partially as a result of the precise or imprecise excision, respectively, of the P-element (158, 159, 177). Although females flies homozygous for the revertant alleles were viable, those carrying one copy of the revertant allele and one copy of *L5* (a null allele of *RpII215*) showed decreased viability, presumably because they synthesize suboptimal levels of RNAPII (158, 159, 177). These revertant alleles should prove useful in analyses of the physiological and developmental consequences of a decrease in the level of assembled RNAPII.

CONCLUDING REMARKS

We have seen how genetics, molecular genetics, and biochemistry have colluded in speeding the advancement of knowledge of RNAPs. Perhaps the most important advance has been the realization of the extent to which RNAPs and processes of transcription are fundamentally the same in eukaryotes and prokaryotes. This emphasizes the value of playing off the findings from one organism against those from another in reaching an understanding of the fundamental principles of transcription. Most research so far has been done with the largest subunits of RNAP; their role in transcription initiation, elongation, and termination, as well as in enzyme assembly and stability, is clearly indicated. However, the function of the additional subunits in eukaryotic RNAPs is largely unknown; especially intriguing are the ones that are common to all three eukaryotic enzymes. In *S. cerevisiae*, five subunits are common to all three RNAPs and the genes encoding them are essential for cellular growth, a result suggesting that these subunits play an essential role in transcription. The observation that these subunits do not appear to have any prokaryotic counterparts suggests that they do not play an essential role in the basic RNA catalysis process but are involved in functions that are specific to eukaryotes and that are performed by all three classes of RNAPs. Suggestions include nuclear localization, enzyme stability, coordinate regulation of rRNA, mRNA and tRNA synthesis (185), and interaction with regulatory factors or with chromatin components. The isolation and characterization of mutations in the genes that encode these subunits have already started.

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