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edited by

WALDO E. COHN KIVIE MOLDAVE

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Abbreviations and Symbols

All contributors to this Series are asked to use the terminology (abbreviations and symbols) recommended by the IUPAC-IUB Commission on Biochemical Nomenclature (CBN) and approved by IUPAC and IUB, and the Editor endeavors to assure conformity. These Recommendations have been published in many journals (*1*, *2*) and compendia (*3*) in four languages and are available in reprint form from the Office of Biochemical Nomenclature (OBN), as stated in each publication, and are therefore considered to be generally known. Those used in nucleic acid work, originally set out in section 5 of the first Recommendations (*1*) and subsequently revised and expanded (*2*, *3*), are given in condensed form (I–V) below for the convenience of the reader. Authors may use them without definition, when necessary.

I. Bases, Nucleosides, Mononucleotides

1. *Bases* (in tables, figures, equations, or chromatograms) are symbolized by Ade, Gua, Hyp, Xan, Cyt, Thy, Oro, Ura; Pur = any purine, Pyr = any pyrimidine, Base = any base. The prefixes S–, H_2, F–, Br, Me, etc., may be used for modifications of these.

2. *Ribonucleosides* (in tables, figures, equations, or chromatograms) are symbolized, in the same order, by Ado, Guo, Ino, Xao, Cyd, Thd, Ord, Urd (Ψrd), Puo, Pyd, Nuc. Modifications may be expressed as indicated in (1) above. Sugar residues may be specified by the prefixes r (optional), d (=deoxyribo), a, x, l, etc., to these, or by two three-letter symbols, as in Ara-Cyt (for aCyd) or dRib-Ade (for dAdo).

3. *Mono-, di-, and triphosphates of nucleosides* (5′) are designated by NMP, NDP, NTP. The N (for "nucleoside") may be replaced by any one of the nucleoside symbols given in II-1 below. 2′-, 3′-, and 5′- are used as prefixes when necessary. The prefix d signifies "deoxy." [Alternatively, nucleotides may be expressed by attaching P to the symbols in (2) above. Thus: P-Ado = AMP; Ado-P = 3′-AMP] cNMP = cyclic 3′:5′-NMP; Bt_2cAMP = dibutyryl cAMP, etc.

II. Oligonucleotides and Polynucleotides

1. Ribonucleoside Residues

(a) Common: A, G, I, X, C, T, O, U, Ψ, R, Y, N (in the order of I-2 above).

(b) Base-modified: sI or M for thioinosine = 6-mercaptopurine ribonucleoside; sU or S for thiouridine; brU or B for 5-bromouridine; hU or D for 5,6-dihydrouridine; i for isopentenyl; f for formyl. Other modifications are similarly indicated by appropriate *lower-case* prefixes (in contrast to I-1 above) (*2*, *3*).

(c) Sugar-modified: prefixes are d, a, x, or l as in I-2 above; alternatively, by *italics* or boldface type (with definition) unless the entire chain is specified by an appropriate prefix. The 2′-*O*-methyl group is indicated by *suffix* m (e.g., -Am- for 2′-*O*-methyladenosine, but -mA- for 6-methyladenosine).

(d) Locants and multipliers, when necessary, are indicated by superscripts and subscripts, respectively, e.g., -m_2^6A- = 6-dimethyladenosine; -s^4U- or -^{4}S- = 4-thiouridine; -ac^{4}Cm- = 2′-*O*-methyl-4-acetylcytidine.

(e) When space is limited, as in two-dimensional arrays or in aligning homologous sequences, the prefixes may be placed *over the capital letter*, the suffixes *over the phosphodiester symbol*.

2. Phosphoric Residues [left side = 5′, right side = 3′ (or 2′)]

(a) Terminal: p, e.g., pppN . . . is a polynucleotide with a 5′-triphosphate at one end; Ap is adenosine 3′-phosphate; C > p is cytidine 2′:3′-cyclic phosphate (*1*, *2*, *3*); p < A is adenosine 3′:5′-cyclic phosphate.

(b) Internal: hyphen (for known sequence), comma (for unknown sequence); unknown sequences are enclosed in parentheses. E.g., pA-G-A-C(C_2,A,U)A-U-G-C > p is a sequence with a (5′) phosphate at one end, a 2′:3′-cyclic phosphate at the other, and a tetranucleotide of unknown sequence in the middle. **(Only codon triplets should be written without some punctuation separating the residues.)**

3. Polarity, or Direction of Chain

The symbol for the phosphodiester group (whether hyphen or comma or parentheses, as in 2b) represents a 3′-5′ link (i.e., a 5′ . . . 3′ chain) unless otherwise indicated by appropriate numbers. "Reverse polarity" (a chain proceeding from a 3′ terminus at left to a 5′ terminus at right) may be shown by numerals or by right-to-left arrows. Polarity in any direction, as in a two-dimensional array, may be shown by appropriate rotation of the (capital) letters so that 5′ is at left, 3′ at right when the letter is viewed right-side-up.

4. Synthetic Polymers

The complete name or the appropriate group of symbols (see II-1 above) of the repeating unit, **enclosed in parentheses if complex or a symbol,** is either (a) preceded by "poly," or (b) followed by a subscript "n" or appropriate number. **No space follows "poly"** (2, 5).

The conventions of II-2b are used to specify known or unknown (random) sequence, e.g.,

polyadenylate = poly(A) or A_n, a simple homopolymer;

poly(3 adenylate, 2 cytidylate) = poly(A_3C_2) or $(A_3,C_2)_n$, an *irregular* copolymer of A and C in 3 : 2 proportions;

poly(deoxyadenylate-deoxythymidylate) = poly[d(A-T)] or poly(dA-dT) or $(dA\text{-}dT)_n$ or $d(A\text{-}T)_n$, an ***alternating*** copolymer of dA and dT;

poly(adenylate,guanylate,cytidylate,uridylate) = poly(A,G,C,U) or $(A,G,C,U)_n$, a random assortment of A, G, C, and U residues, proportions unspecified.

The prefix copoly or oligo may replace poly, if desired. The subscript "n" may be replaced by numerals indicating actual size, e.g., $A_n \cdot dT_{12\text{-}18}$.

III. Association of Polynucleotide Chains

1. *Associated* (e.g., H-bonded) chains, or bases within chains, are indicated by a *center dot* (not a hyphen or a plus sign) separating the *complete* names or symbols, e.g.:

poly(A) · poly(U) or $A_n \cdot U_m$

poly(A) · 2 poly(U) or $A_n \cdot 2U_m$

poly(dA-dC) · poly(dG-dT) or $(dA\text{-}dC)_n \cdot (dG\text{-}dT)_m$.

2. *Nonassociated* chains are separated by the plus sign, e.g.:

$2[\text{poly(A)} \cdot \text{poly(U)}] \rightarrow \text{poly(A)} \cdot 2\ \text{poly(U)} + \text{poly(A)}$

or $2[A_n \cdot U_m] \rightarrow A_n \cdot 2U_m + A_n$.

3. Unspecified or unknown association is expressed by a comma (again meaning "unknown") between the completely specified chains.

Note: In all cases, each chain is completely specified in one or the other of the two systems described in II-4 above.

IV. Natural Nucleic Acids

RNA	ribonucleic acid or ribonucleate
DNA	deoxyribonucleic acid or deoxyribonucleate
mRNA; rRNA; nRNA	messenger RNA; ribosomal RNA; nuclear RNA
hnRNA	heterogeneous nuclear RNA
D-RNA; cRNA	"DNA-like" RNA; complementary RNA

mtDNA	mitochondrial DNA
tRNA	transfer (or acceptor or amino-acid-accepting) RNA; replaces sRNA, which is not to be used for any purpose
aminoacyl-tRNA	"charged" tRNA (i.e., tRNA's carrying aminoacyl residues); may be abbreviated to AA-tRNA
alanine tRNA or $tRNA^{Ala}$, etc.	tRNA normally capable of accepting alanine, to form alanyl-tRNA, etc.
alanyl-tRNA or alanyl-$tRNA^{Ala}$	The same, with alanyl residue covalently attached. [*Note:* fMet = formylmethionyl; hence $tRNA^{fMet}$, identical with $tRNA_f^{Met}$]

Isoacceptors are indicated by appropriate subscripts, i.e., $tRNA_1^{Ala}$, $tRNA_2^{Ala}$, etc.

V. Miscellaneous Abbreviations

P_i, PP_i	inorganic orthophosphate, pyrophosphate
RNase, DNase	ribonuclease, deoxyribonuclease
t_m (not T_m)	melting temperature (°C)

Others listed in Table II of Reference 1 may also be used without definition. No others, with or without definition, are used unless, in the opinion of the editor, they increase the ease of reading.

Enzymes

In naming enzymes, the 1978 recommendations of the IUB Commission on Biochemical Nomenclature (*4*) are followed as far as possible. At first mention, each enzyme is described *either* by its systematic name *or* by the equation for the reaction catalyzed *or* by the recommended trivial name, followed by its EC number in parentheses. Thereafter, a trivial name may be used. Enzyme names are not to be abbreviated except when the substrate has an approved abbreviation (e.g., ATPase, but not LDH, is acceptable).

References

1. *JBC* **241**, 527 (1966); *Bchem* **5**, 1445 (1966); *BJ* **101**, 1 (1966); *ABB* **115**, 1 (1966), **129**, 1 (1969); and elsewhere.†
2. *EJB* **15**, 203 (1970); *JBC* **245**, 5171 (1970); *JMB* **55**, 299 (1971); and elsewhere.†
3. "Handbook of Biochemistry" (G. Fasman, ed.), 3rd ed. Chemical Rubber Co., Cleveland, Ohio, 1970, 1975, Nucleic Acids, Vols. I and II, pp. 3–59.
4. "Enzyme Nomenclature" [Recommendations (1978) of the Nomenclature Committee of the IUB]. Academic Press, New York, 1979.
5. "Nomenclature of Synthetic Polypeptides," *JBC* **247**, 323 (1972); *Biopolymers* **11**, 321 (1972); and elsewhere.†

Abbreviations of Journal Titles

Journals	*Abbreviations used*
Annu. Rev. Biochem.	ARB
Annu. Rev. Genet.	ARGen
Arch. Biochem. Biophys.	ABB
Biochem. Biophys. Res. Commun.	BBRC
Biochemistry	Bchem
Biochem. J.	BJ
Biochim. Biophys. Acta	BBA
Cold Spring Harbor	CSH

Cold Spring Harbor Lab.	CSHLab
Cold Spring Harbor Symp. Quant. Biol.	CSHSQB
Eur. J. Biochem.	EJB
Fed. Proc.	FP
Hoppe-Seyler's Z. physiol. Chem.	ZpChem
J. Amer. Chem. Soc.	JACS
J. Bacteriol.	J. Bact.
J. Biol. Chem.	JBC
J. Chem. Soc.	JCS
J. Mol. Biol.	JMB
J. Nat. Cancer Inst.	JNCI
Mol. Cell. Biol.	MCBiol
Mol. Cell. Biochem.	MCBchem
Mol. Gen. Genet.	MGG
Nature, New Biology	Nature NB
Nucleic Acid Research	NARes
Proc. Nat. Acad. Sci. U.S.	PNAS
Proc. Soc. Exp. Biol. Med.	PSEBM
Progr. Nucl. Acid. Res. Mol. Biol.	This Series

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Gene Conversion in Trypanosome Antigenic Variation

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I. Summary

Antigenic variation allows African trypanosomes[1] to escape the immune defenses of their hosts. This occurs through differential surface antigen gene activation, with only one antigen gene being expressed at any one time among a large repertoire of different se-

[1] Abbreviations: The *nomenclature* of the trypanosome antigenic types follows the system recommended by Lumsden [W. H. R. Lumsden, *System. Parasitol.* **4**, 373 (1982)]. It is based on the name of the place where the immunological characterization of the antigen repertoire was performed. For instance, AnTat 1.10 stems from *An*twerp *T*rypanozoon *a*ntigenic *t*ype number 10, from the repertoire number 1.

BC = *B*asic *C*opy of the antigen gene: it is the template used for the synthesis of the additional, expression-linked gene copy. The BC is the donor in the gene conversion process.

ELC = *E*xpression-*L*inked *C*opy of the antigen gene: it is the copy synthesized in the clone where the gene is activated.

kb = Kilobase pairs.

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quences. The differential gene activation can be achieved by gene conversion between antigen-specific sequences, taking place in a telomeric gene expression site. The extent of the gene conversion event may vary considerably (from 1 to more than 40 kb), although it is generally about 3 kb. The gene conversion size seems to depend on the extent of homology between the donor and target sequences involved in the recombinational process. Analysis of several gene conversion endpoints, and comparison with the mechanism for mating type interconversion in yeast, suggests that the process could be triggered by a cut upstream from the antigen gene, then resolved by a crossing-over in a region of homology downstream from this point. This crossing-over could take place anywhere within the regions of homology, but only the recombinations leading to the successful generation of new antigen-coding sequences would be selected. Based on this gene conversion model, it is possible to explain the generation of "mini-chromosomes" harboring antigen-specific sequences. Those could represent residual "targets" carrying a replication origin. Translocation of such haploid telomeric sequences to the other end of other chromosomes would lead to internalization of formerly telomeric genes, and a clustering of haploid antigen-specific sequences near the chromosome termini. I suggest that the orientation of the conversion could be determined by the chromatin structure around the antigen gene. Actively transcribed chromatin indeed exhibits an "open" configuration that may be more susceptible to cutting by the putative site-specific endonuclease catalyzing the gene conversion event. This hypothesis implies that, among all antigen-specific sequences, the potentially transcribable ones could be preferential targets for gene conversion.

The existence of such preferred target sequences has been postulated to be essential for the generation of polymorphism by gene conversion in multigene families (*1*, *2*). In combination with another gene activation mechanism that does not depend on gene duplication, gene conversion leads to rapid modifications of the trypanosome surface antigen repertoire, by gain, loss and alterations of different sequences, and by changes in their activation rate. Sexual conjugation between trypanosomes containing different copy numbers of antigen-specific sequences further leads to evolution of the antigen gene repertoire.

II. Antigenic Variation Is due to Differential Gene Expression

African trypanosomes are parasitic flagellates that grow alternately in two successive hosts: first *Glossina* flies and then various mammals,

including man. At the end of their development in the fly salivary glands, the trypanosomes completely surround themselves with a thick layer of a single, densely packed glycoprotein of about 65,000 Da. Following insect bite, the trypanosomes invade the bloodstream of a mammal, where the protein part of their surface molecule is recognized as an antigen by the immune system of the host. The antibody response leads to a rapid destruction of most cells in this first parasite population, but antigenic variation allows a few individuals (less than 0.1%) to survive. Some of them can undergo proliferation into new parasitemia waves, characterized by different antigenic specificities. The interplay between antigenic variation and immune response gives rise to the chronic infection characteristic of the African trypanosomiases (for reviews on trypanosome antigenic variation and antigen structure, see *3–7*).

The repertoire of different antigenic types in each cell consists of at least 100 antigens (*8*), and it has been established that to this large antigen repertoire corresponds a similar collection of different antigen genes (*9–12*). Only a single antigen is synthesized by a given cell, due to transcriptional control of gene expression (*13–16*). Chronic infection by African trypanosomes is thus maintained by the differential and successive expression of a large number of different antigen genes.

The expression of these genes is not random, since some antigenic types preferentially appear early in the chronic infection (predominant types), whereas others are only rarely observed (late types) (*17–21*). Moreover, in the fly salivary glands, only a small, characteristic fraction of the total antigen repertoire is expressed (metacyclic types) (*22, 23*). These observations point to a possible programming, or induction of antigen gene expression. However, at least in bloodstream trypanosomes, no inducer for antigenic variation has so far been found; in particular, antibodies do not seem to be involved in this mechanism, since antigenic variation has been reported to occur in immunosuppressed mice (*24*), or *in vitro* (*25*).

III. Antigen Gene Expression Is Often Linked to DNA Rearrangements

The use of specific cDNA probes (*10, 15, 16, 26*) has allowed the study of the expression of several variant-specific antigen genes. In different cases, genome rearrangements were clearly involved in antigen gene expression: it was indeed found that some of the specific antigen mRNAs are synthesized on an additional copy (ELC, for expression-linked copy) of the gene (BC, for basic copy), after transposi-

tion of the ELC into an expression site distinct from the BC environment (*12, 14, 27–35*). However in other cases, no DNA rearrangement could specifically be linked to the antigen gene expression (*26, 36–39*). The trypanosome variant-specific antigen genes can thus be activated by at least two different mechanisms (*39–44*).

Initial reports for genome rearrangements unlinked to antigen gene expression (*26*) were in fact due to the telomeric location of many of these genes or related sequences (*28, 29, 34, 45–48*). Indeed many antigen-specific sequences are found near chromosome ends and the terminal size of the telomeres is continuously changing. A constant length increase, probably linked to the mechanism of DNA replication in chromosome ends (*49, 50*) is corrected by occasional shortenings whose extent and frequency differ between transcriptionally active and inactive telomeres (*49, 51*). These DNA alterations do not seem to be involved in antigenic variation, although telomere terminal shortenings have often, but not always, been observed in connection with the telomere involvement in antigen gene expression (*51*).

It is worth stressing here that the trypanosome antigen genes are always transcribed in a telomere, whatever their activation mechanism may be. In particular, the genes activated without apparent DNA rearrangement have always been found to be telomeric (*37, 38, 39, 43, 49*). Moreover, non-telomeric antigen-specific sequences have never been found to be transcribed. The meaning of these observations is completely unknown; at least they point to the need of a telomeric location for antigen genes to be expressed.

IV. Evidence for Gene Conversion as a Mechanism for Antigenic Variation

The additional copy (ELC) of variant-specific antigen genes can be transposed into an expression site that seems identical in different trypanosome clones from the same stock (*28, 29, 31, 34*). This observation suggests that at each antigenic switch, the successive ELCs are replacing each other in the same site, driving out the formerly expressed gene copy. This hypothesis found support in the observation that a sequence at the 3′ end of the gene is shared by different antigen coding sequences (*52–55*); thus this sequence might be considered as one of the recognition sequences involved in the recombinational processes of ELC replacement (*9, 28, 30, 31, 56*). Similarly, there is indirect evidence that a repeat located upstream from the antigen gene may be required for ELC transposition, at least in some cases (*9, 28, 31, 57, 58*).

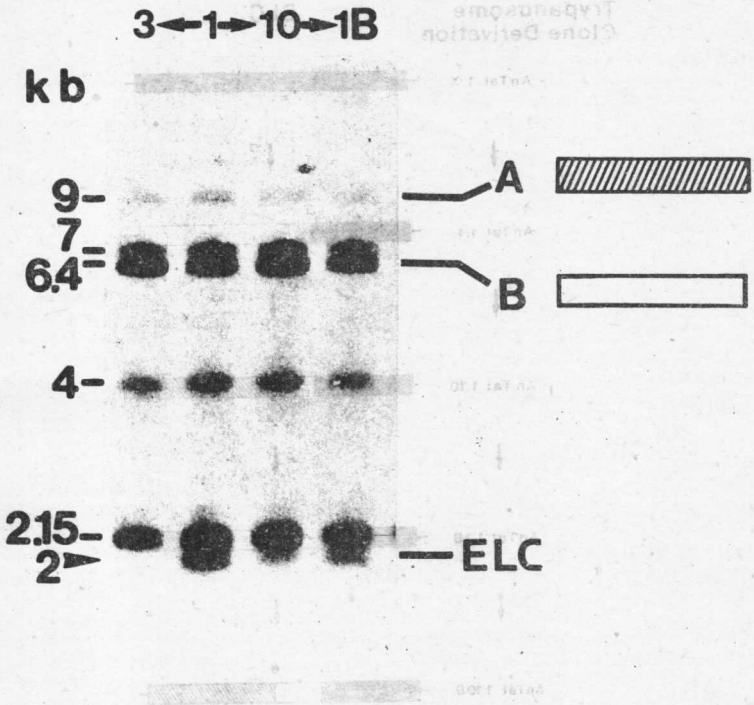

FIG. 1. The *T. brucei* AnTat 1.1 gene family. The five AnTat 1.1-specific sequences were separated by electrophoresis in 0.85% agarose gel after *Pst*I digestion; as compared with the genomic DNA of the clone expressing the AnTat 1.3 antigenic type (first lane), the DNAs from the clones expressing the AnTat 1.1, 1.10 and 1.1B antigenic types contain an additional AnTat 1.1-specific sequence (ELC, designated by an arrowhead), which is the one transcribed (27, 59). Sequences A and B are involved in the ELC modifications from AnTat 1.1 to 1.1B (see text); they are represented in Fig. 2 by shaded and open boxes, respectively.

A proof for this ELC replacement hypothesis was provided by the analysis of the antigen gene expressed in the *T. b. brucei* AnTat 1.1 clone (AnTat for Antwerp Trypanozoon antigenic type), as well as in two clones successively derived from AnTat 1.1, namely AnTat 1.10 and 1.1B (59). The BCs for the AnTat 1.1 and 1.10 ELCs were found to be two members of the same multigene family (Fig. 1). Interestingly, both are located in telomeric regions (59). The way in which the ELCs synthesized on these templates replace each other in the expression site during AnTat 1.1 to 1.1B clone switching is summarized in Fig. 2.