1982 Supplement to DNA Replication

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DNA Replication

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Preface

DNA Replication (1980) embraces the biochemical, genetic, and physiological aspects of the process and the numerous DNA transactions that determine the structure and function of genetic material. The text and figures were designed to orient and inform students beginning their study of DNA replication as well as professionals already working on the subject. The biochemical emphasis is evident in the devotion of half the book to key enzymes and proteins (polymerases, ligases, nucleases, binding proteins) and the remainder to the cellular mechanisms in which they are employed (replication, repair, recombination, viral life cycles).

Progress in DNA replication has continued at a pace that demands a major addendum after only two years. This 1982 Supplement cites items and key references that alert the reader to important developments from June 1979 to January 1982. It does not repeat the essential facts, figures, ideas, and references in *DNA Replication*. This book is therefore a biennial news summary and not a compendium of *DNA Replication*. The surprising size of this Supplement stems both from the volume of advances in DNA structure, enzymology, and functions and from a wish to recount these advances intelligibly (with numerous pictures and references) rather than telegraphically. In addition, each of the seventeen chapters opens with an abstract, highlights selected with considerable editorial license.

In many special areas I have relied on the knowledge and enthusiastic help of colleagues. Among them, I want to cite especially Roger Kornberg for DNA structure, Mary Ellen Jones and Thomas Traut for biosynthesis of nucleotides, Peter Reichard for the latter and papovaviruses, Robert Lehman for DNA polymerases and recombination, Charles McHenry for DNA polymerase III, David Korn for DNA polymerases α and β, Michael Chamberlin for RNA polymerases, Olke Uhlenbeck for ligases, Nicholas Cozzarelli and Joseph Coleman for gyrases and binding proteins, Stuart Linn for nucleases, Claude Paoletti for inhibitors, Gordon Lark for control of replication, George Stark for gene amplification, Robert Webster for phage M13, Bruce Alberts for phage T4, Charles Richardson for phage T7, David Clayton for mitochondria, Donald Helinski for plasmids, Jerard Hurwitz for adenoviruses, Michael Bishop and Inder Verma for retroviruses, Mehran Goulian for parvoviruses, Bernard Roizman for herpes viruses, William Robinson for hepadna viruses, Philip Hanawalt for DNA repair, Harrison Echols, Charles Radding, Robert Lehman, and Dale Kaiser for recombination, Leslie Orgel for prebiotic oligonucleotide synthesis, and Roberto Crea for chemical synthesis of DNA.

To all these colleagues, I and others who want to be au courant with DNA transactions are indebted. I take the responsibility, however, for editorial selections, revisions, and rather substantial additions to give the Supplement a uniform style. I am deeply grateful to Neil Patterson and John Staples of W. H. Freeman and Company for their eager acceptance and skillful execution of this experiment in publishing, to Betty Bray for typing this manuscript from crude handwritten copy, to Charlene Levering for artistic insights that enhanced the illustrations, to Patricia Brewer for excellent editorial styling, and to Roger Kornberg and Leroy Bertsch for careful review of the entire manuscript.

January 1982 *Arthur Kornberg*

Contents

S1

Structure and Functions of DNA*

Abstract

The past two years have been the most eventful in the history of DNA structure since the discovery of the double helix. X-ray crystallographic analysis, made possible only by the efficient new technologies of oligodeoxyribonucleotide synthesis, provides for the first time the structural details at individual base pairs along the helix. Cloning of long DNA molecules engineered to contain these synthetic sequences in defined arrangements further permits their natural behavior in solution to be analyzed. The x-ray crystal structures of DNA molecules so far determined have given several striking results: The essential features of A-DNA and B-DNA, as proposed and refined by fiber diffraction analysis, were confirmed;

*Section numbers from *DNA Replication* are listed below, with an S preceding those for which new information is provided in this Supplement.

novel properties of B-DNA, with regard to nucleotide conformation and flexibility of the double helix, have emerged; and a new family of DNA structures, termed Z-DNA, was discovered, which may hold clues to the control of gene expression.

S1-1 DNA: Past and Present

Since *DNA Replication* appeared two years ago, the major currents of DNA research have continued and in several places have become much clearer. The ease of isolating, analyzing, synthesizing, and rearranging DNA sequences and genes and the ability to insert these recombinant DNAs into cells have fueled a stampede from all quarters of biology and has titillated chemists and industrialists. Interest in DNA transactions such as replication, repair, transposition, and viral multiplication has increased but remains predominantly focused on intact cellular systems. Too little attention has been given to identifying the proteins that translate the genetic message into cellular action, the agents for creating and controlling all genetic events. Still, as this Supplement will illustrate, progress in many areas has provided insights into the nature of DNA replication that impress all of us who observe the advancing front of DNA knowledge.

S1-3 A Double Helical Structure[1-5]

Three families of DNA structure are now recognized, and examples of all three have been crystallized and subjected to x-ray structure determination (Fig. S1-1). The B family is the predominant form in solution. The crystal structure shows a classical, right-handed Watson–Crick double helix, with a striking degree of flexibility and sequence dependence of certain helix parameters. Conversion of the B structure to the A and Z forms usually attends lowering of water activity, for example, by addition of ethanol or at low humidity or high salt concentration, although some DNAs may adopt the Z structure in physiological conditions. A remarkable feature of Z-DNA is the left-handed sense of the double helix.

1. Viswamitra, M. A., Kennard, O., Shakked, Z., Jones, P. G., Sheldrick, G. M., Salisbury, S., and Falvello, L. (1978) *Nat. 273*, 687.
2. Wang, A. H.-J., Quigley, G. J., Kolpak, F. J., Crawford, J. L., van Boom, J. H., van der Marel, G., and Rich, A. (1979) *Nat. 282*, 680.
3. Drew, H., Takano, T., Tanaka, S., Itakura, K., and Dickerson, R. E. (1980) *Nat. 286*, 567.
4. Wing, R., Drew, H., Takano, T., Broka, C., Tanaka, S., Itakura, K., and Dickerson, R. E. (1980) *Nat. 287*, 755.
5. Dickerson, R. E., Drew, H. R., Conner, B. M., Wing, R. M., Fratini, A. V., and Kopka, M. L. (1982) *Science 216*, 475.

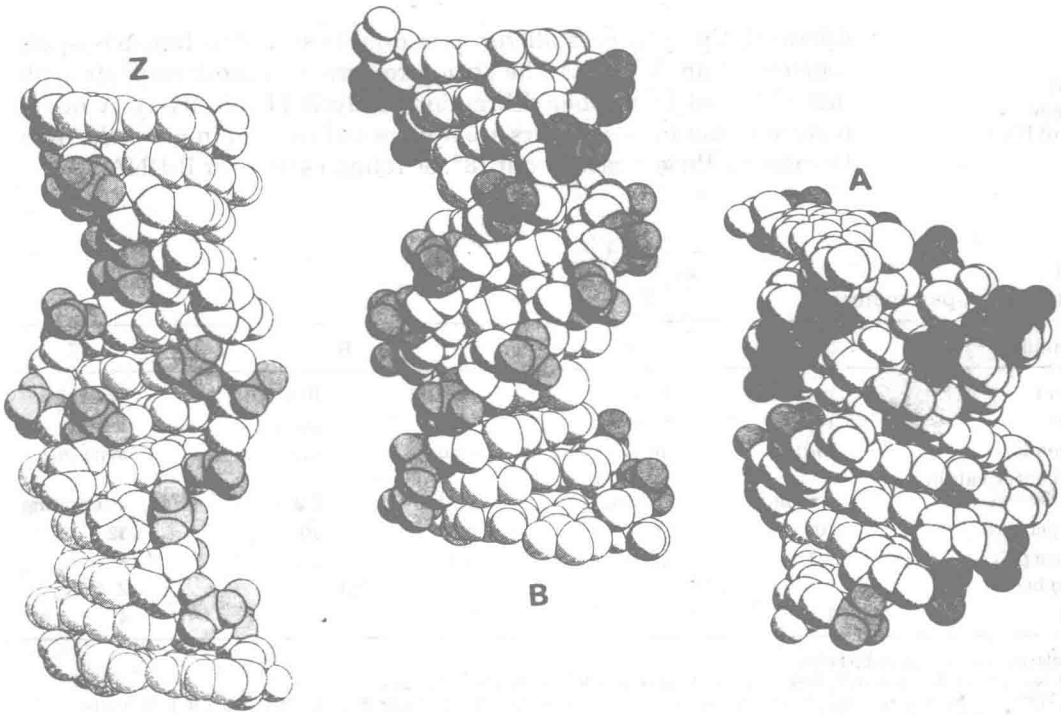

FIGURE S1-1
Space-filling drawings of A-, B-, and Z-DNA. Uniform-scale comparisons are made of equal lengths based on single-crystal structure analyses of A-DNA, three stacked CCGG tetramers; B-DNA, one CGCGAATTCGCG molecule; Z-DNA, three stacked CGCG tetramers. The tetramers have not been adjusted; they are stacked atop one another with the proper helical repeat, hence the phosphates needed to bridge between tetramers are absent. (Courtesy of Professor R. E. Dickerson.)

A-DNA

The A structure is found in aggregates of DNA induced by 70–75 percent ethanol[6,7] and in fibers of DNA in a dehydrated state. The A structure is also the principal conformation of RNA under all conditions. (Polyribonucleotides, whether in an RNA–RNA duplex or a DNA–RNA heteroduplex, are unable to adopt the B conformation due to steric hindrance between the ribose 2′ hydroxyl group and the phosphate group of the adjacent nucleotide.) The tetranucleotide

6. Zimmerman, S. B., and Pheiffer, B. H. (1979) *JMB 135*, 1023.
7. Gray, D. M., Edmondson, S. P., Lang, D., and Vaughan, M. (1979) *N. A. Res. 6*, 2089.

d(iodo-CpCpGpG) crystallizes as a double-stranded four-base-pair segment of an A helix.[8] The structure agrees in most respects with that obtained from fiber diffraction analysis (Table S1-1). A novel feature is that the base pairs are not flat but twisted in propeller-like fashion, on the average about 18°, as found earlier for B-DNA.

TABLE S1-1
Nucleic acid helix parameters

Family	A		B		Z
Environment	crystal	fiber	crystal	fiber	crystal
Helix sense	right	right	right	right	left
Glycosyl bonds	*anti*	*anti*	*anti*	*anti*	*syn/anti*
Sugar ring conformation (pucker)	C3′-*endo*	C3′-*endo*	variable	C2′-*endo*	alternating
Base pairs per turn	10.7	11	9.7[a]	10	12
Rise per base pair (Å)	2.3	2.6	3.3[a]	3.4	3.7
Family members	A,A′		B,C		Z,Z′
References	1	2	3	2	4

[a]Average of eleven values for a 12-bp helix.

1. Conner, B. N., Takano, T., Tanaka, S., Itakura, K., and Dickerson, R. E. (1981) *Nat. 295,* 294.
2. Arnott, S. (1976) in *Organization and Expression of Chromosomes* (Allfrey, V. G., Bautz, E. F. K., McCarthy, B. J., Schimke, R. T., and Tissieres, A., eds.) Dahlem Conference, Berlin, p. 209.
3. Drew, H. R., Wing, R. M., Takano, T., Broka, C., Tanaka, S., Itakura, K., and Dickerson, R. E. (1981) *PNAS 78,* 2179.
4. Wang, A. H.-J., Quigley, G. J., Kolpak, F. J., Crawford, J. L. van Boom, J. H., van der Marel, G., and Rich, A. (1979) *Nat. 282,* 680.

B-DNA

A representative of the B family of structures, the principal conformation of DNA in solution, has been obtained in crystals of the dodecamer d(CpGpCpGpApApTpTpCpGpCpG).[9] This molecule is self-complementary and in duplex form contains a site for cleavage by the restriction endonuclease *Eco*RI. The structure of the duplex at 1.9 Å resolution[10] corresponds closely with the classical B helix deduced from fiber diffraction studies (Table S1-1). Minor differences from classical B helix geometry were expected: A propeller twist of the base pairs and smooth bending of the helix axis were proposed

8. Conner, B. N., Takano, T., Tanaka, S., Itakura, K., and Dickerson, R. E. (1981) *Nat. 295,* 294.
9. Wing, R., Drew, H., Takano, T., Broka, C., Tanaka, S., Itakura, K., and Dickerson, R. E. (1980) *Nat. 287,* 755; Dickerson, R. E., and Drew, H. R. (1981) *JMB 149,* 761; Drew, H. R., and Dickerson, R. E. (1981) *JMB 151.* 535; Dickerson, R. E., and Drew, H. R. (1981) *PNAS 78,* 7318.
10. Drew, H. R., Wing, R. M., Takano, T., Broka, C., Tanaka, S., Itakura, K., and Dickerson, R. E. (1981) *PNAS 78,* 2179.

on the basis of energy minimization calculations;[11] flexibility with regard both to bending of the helix axis and to the rotation from one base pair to the next around the helix axis was indicated by physicochemical measurements on DNA in solution.[12] These expectations are borne out in the dodecamer structure.

The base pairs are twisted in propeller fashion from 5 to 19°, presumably because such twisting increases the overlap of a base with those above and below it in the same polynucleotide chain. The helix axis is bent, smoothly and without kinking, by a total of 19° over the full length of the dodecamer. This bending is probably a consequence of the packing arrangement in the crystal and not inherent in the dodecamer, but it illustrates the ease and mechanism of bending B-DNA. The rotation per base pair in the dodecamer ranges from 32 to 45°, with a mean of 37°, corresponding to 9.7 base pairs per turn of the helix (which may be compared with a value of 10.6 base pairs per turn estimated for all DNAs in solution except poly dA · poly dT).[13] In contrast with the bending of the helix axis, the nonuniform rotation per base pair seen in the crystal structure probably reflects the state of the dodecamer in solution, since the value of the rotation at a particular base pair step correlates in a striking way with the rate constant for cleavage at that point by DNase I.[14] It remains to be seen whether this sequence-dependent feature of B-DNA structure is recognized by other nucleases as well.

The flexibility and internal variation of the dodecamer structure can be traced to a variable conformation of the sugar-phosphate backbone, especially the deoxyribose ring (Fig. S1-2). A distribution extending from the C3′-*endo* ring conformation characteristic of the classical A helix to the C2′-*endo* conformation of the classical B helix is observed. An extreme case of this variable sugar conformation may be found in the "alternating-B" structure proposed for the alternating copolymer poly d(AT).[15] This proposal is based on the crystal structure of the tetranucleotide d(pApTpApT), which is organized in dinucleotide units, rather than as a single, 4-bp stretch of double helix.[16] The sugar conformation is C3′-*endo* within a unit and C2′-*endo* between units. While there is no direct evidence for the

11. Levitt, M. (1978) *PNAS 75*, 640.
12. Record, M. T., Jr., Mazur, S. J., Melançon, P., Roe, J.-H., Shaner, S. L., and Unger, L. (1981) *ARB 50*, 997.
13. Wang, J. C. (1979) *PNAS 76*, 200; Peck, L. J., and Wang, J. C. (1981) *Nat. 292*, 375; Rhodes, D., and Klug, A. (1981) *Nat. 292*, 378.
14. Lomonossoff, G. P., Butler, P. J. G., and Klug, A. (1981) *JMB 149*, 745.
15. Klug, A., Jack, A., Viswamitra, M. A., Kennard, O., Shakked, Z., and Steitz, T. A. (1979) *JMB 131*, 669.
16. Viswamitra, M. A., Kennard, O., Shakked, Z., Jones, P. G., Sheldrick, G. M., Salisbury, S., and Falvello, L. (1978) *Nat. 273*, 687.

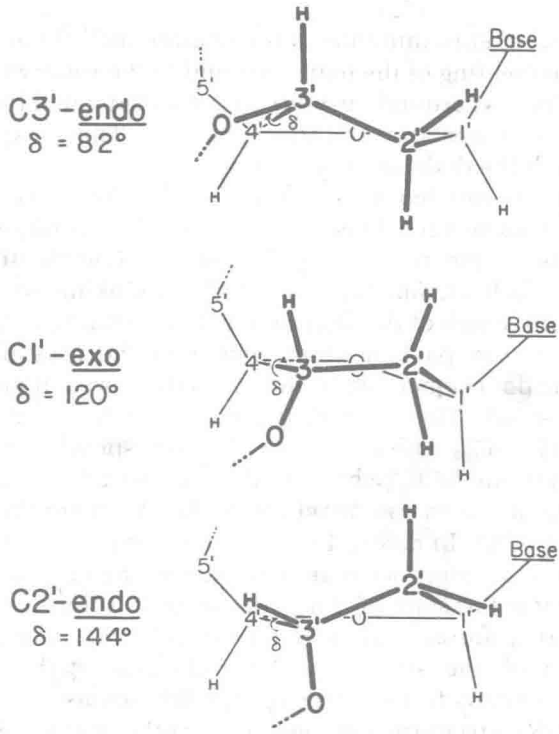

FIGURE S1-2
Relationship between deoxyribose sugar puckering and the C5′-C4′-C3′-03′ main chain torsion angle δ. This torsion angle is a much more sensitive and easily observed measure of sugar conformation than the appearance of the 5-membered ring itself. (Courtesy of Professor R. E. Dickerson.)

alternating-B structure of poly d(AT) copolymer in solution, such a structure would explain why digestion of the copolymer to the limit with DNase I results in a dinucleotide product.[17]

Z-DNA

The proposed alternating-B structure foreshadowed the discovery of an entirely new family of DNA structures in which sugar conformations alternate between C2′-*endo* and C3′-*endo* or C1′-*exo*. The first members of this new family, termed Z-DNA because of the zigzag course of the backbone, were found by x-ray crystal-

17. Scheffler, I. E., Elson, E. L., and Baldwin, R. L. (1968) *JMB 36*, 291.

lographic analysis of the hexanucleotide d(CpGpCpGpCpG)[18] and the tetranucleotide d(CpGpCpG).[19] The structures of the duplex forms of these molecules are, like that of d(pApTpApT), based on a dinucleotide unit, but they differ from A-, B-, and alternating-B DNA in two fundamental respects. First, the helix sense is left- rather than right-handed. Second, the glycosyl bonds alternate between *syn* and *anti* orientations, in contrast with the all-*anti* conformation of previously studied DNAs.

To what extent might the Z structure occur in nature in DNA sequences of interest? It can be argued that the Z structure is limited to alternating purine–pyrimidine sequences, because steric repulsion prevents a pyrimidine from adopting the *syn* conformation.[20] Moreover, a Z-compatible sequence may be forced into the B structure by a neighboring region of B-DNA (as occurs in the dodecamer discussed above, which is found in the B structure in spite of the tendency of eight of twelve residues to form a Z helix on their own in the same conditions). Factors that might favor the Z structure include methylation[21,22] and specific DNA-binding proteins. The search for Z-DNA in a physiologically important context should be facilitated by the isolation of a remarkably specific antibody that recognizes only the Z form of DNA.[23] The antibodies bind specifically and regularly to interband regions of polytene chromosomes of *Drosophila*.[24] Because cloned segments of poly d(GC) in plasmids undergo a reversible B to Z transformation,[25] it seems possible that similar changes in conformation in chromosomes may accompany local environmental influences and have far-reaching effects.

S1-6 Shape

The DNA helix behaves as a stiff rod only for short segments in the size range of the persistence length (about 500 Å, or 150 base pairs).[26] Even such short DNA molecules show a finite probability of bending: Restriction fragments as short as 242 base pairs have been closed

18. Wang, A. H.-J., Quigley, G. J., Kolpak, F. J., Crawford, J. L., van Boom, J. H., van der Marel, G., and Rich, A. (1979) *Nat. 282*, 680.
19. Drew, H., Takano, T., Tanaka, S., Itakura, K., and Dickerson, R. E. (1980) *Nat. 286*, 567; Drew, H. R., and Dickerson, R. E. (1981) *JMB 152*, 723.
20. Drew, H., Takano, T., Tanaka, S., Itakura, K., and Dickerson, R. E. (1980) *Nat. 286*, 567.
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22. Möller, A., Nordheim, A., Nichols, S. R., and Rich, A. (1981) *PNAS 78*, 4777.
23. Lafer, E. M., Möller, A., Nordheim, A., Stollar, B. D., and Rich, A. (1981) *PNAS 78*, 3546.
24. Nordheim, A., Pardue, M. L., Lafer, E. M., Möller, A., Stollar, B. D., and Rich, A. (1981) *Nat. 294*, 417.
25. Klysik, J., Stirdivant, S. M., Larson, J. E., Hart, P. A., and Wells, R. D. (1981) *Nat. 290*, 672; Peck, L., Nordheim, A., Rich, A., and Wang, J. C., personal communication.
26. Hagerman, P. J. (1981) *Biopolymers 20*, 1503.

covalently into circles by T4 DNA ligase.[27] This flexibility of the double helix is also illustrated by the folding of large DNA molecules into compact structures. The tight winding of DNA about a histone octamer to form the nucleosome (Section S9-7) is effected by the binding energies of the strong ionic interactions. Also at very low DNA concentrations, monomolecular condensation can be induced by polyamines[28] or even by the trivalent ion $Co^{3+}(NH_3)_6$.[29] The tightly wound condensates have diameters close to those of the phage head interior.

Evidence for a sequence-specific fold in DNA, the so-called cruciform or double hairpin structure, has recently been found.[30] The supercoiled forms of ColE1, pBR322, and ϕX174 RF DNA all contain one major site of cleavage by the single-strand-specific nuclease S1. In every case this site lies in an inverted repeat region, suggestive of the double hairpin loops previously proposed to accommodate the strain of negative supercoiling with the minimum loss of base pairing. Remarkably, the site of cleavage in ϕX174 DNA is the same as the site of binding of a prepriming replication protein to a single strand of DNA and supports the idea that this protein recognizes a hairpin-loop structure.[31] Hairpin-loop formation by an inverted repeat is a local property that can be transmitted from one supercoiled DNA to another by moving the responsible region through recombinant DNA manipulation.[32]

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27. Shore, D., Langowski, J., and Baldwin, R. L. (1981) *PNAS 78*, 4833.
28. Gosule, L. C., and Schellman, J. A. (1976) *Nat. 259*, 333.
29. Widom, J., and Baldwin, R. L. (1980) *JMB 144*, 431.
30. Lilley, D. M. J. (1980) *PNAS 77*, 6468.
31. Shlomai, J., and Kornberg, A. (1980) *PNAS 77*, 799.
32. Lilley, D. M. J. (1981) *N. A. Res. 9*, 1271.

S2

Biosynthesis of DNA Precursors

Abstract

Purine and pyrimidine biosynthetic pathways and the one-carbon metabolism tha⁴ feeds them are served by multifunctional enzymes and multienzyme complexes that efficiently channel and preserve labile intermediates. New findings include the following: (i) More refined genetic analysis reveals complexities in the regulatory control of the pathways. (ii) Phage-induced replication systems have assemblies that guide even remote DNA precursors directly to the site of polymerization. (iii) Ribonucleotide reduction, a crucial regulatory stage in DNA synthesis, is catalyzed and controlled in mammals much as in *E. coli*. The reductase mechanism, especially the

action of the tyrosyl radical, is under active study. (iv) Inhibition of thymidylate synthesis by methotrexate elevates dUTP to levels that result in harmful incorporation of uracil in DNA. (v) Failure to remove purine nucleotide breakdown products may lead to abnormal salvage, as happens when reduced levels of adenosine deaminase or purine nucleoside phosphorylase produce severe immunodeficiency diseases. However, retention of uric acid, the ultimate purine degradation product in humans, long reviled as the cause of gouty arthritis and renal disease, may prove a friend as a potent antioxidant in combating mutagenic agents that affect aging and cause cancer.

S2-2 Purine Nucleotide Synthesis de Novo

PRPP Synthetase

PRPP synthetase, an enzyme from human erythrocytes, is regulated by the state of its aggregation. In vitro, the enzyme varies in size from a dimer to aggregates of 16 or 32 subunits.[1] Magnesium ATP and other purine nucleotides promote aggregation, while 2,3-bisphosphoglycerate, an inhibitor, promotes dissociation; the smaller forms have only 4 percent of the catalytic activity of the very large.

Genes and Regulation of Purine Biosynthesis

Genes are known in *S. typhimurium* and *E. coli* for all but one of the twelve enzymes that convert PRPP to GMP and AMP (Table S2-1).[2] Most of these genes are physically unlinked, although several respond to a common repressor, isolated on the basis of binding to specific operator regions.[3] ATP and GTP may each act as corepressors with differential effects on reactions before the IMP branchpoint. What once appeared to be a simple and decisive regulation at IMP will likely prove more complex both at this level and at earlier points in the de novo pathway.[4] In yeast, relatively little is known about the

1. Becker, M. A., Meyer, L. J., Huisman, W. H., Lazar, C., and Adams, W. B. (1977) *JBC* 252, 3911; Meyer, L. J., and Becker, M. A. (1977) *JBC* 252, 3919.
2. Bachmann, B. J., and Low, K. B. (1980) *Microbiol. Rev.* 44, 1; Sanderson, K. E., and Hartman, P. E. (1978) *Microbiol. Rev.* 42, 471.
3. Gots, J. S., Benson, C. E., Jochimsen, B., and Koduri, K. R. (1976) in *Microbial Models and Regulatory Elements in the Control of Purine Metabolism*, pp. 23–40, Ciba Foundation Symposium, Purine and Pyrimidine Metabolism; Levine, R. A., and Taylor, M. W. (1982) *J. Bact.* 149, 923; Koduri, K. R., and Gots, J. S. (1980) *JBC* 255, 9594.
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TABLE S2-1
Genes of purine biosynthesis in *E. coli*

Enzyme	Reaction	Gene	Map position[a]
Amidophosphoribosyl-transferase	5-phosphoribosyl-α-pyrophosphate (PRPP) ↓ 5-phosphoribosylamine	*purF*	49
GAR synthetase	↓ glycinamide ribotide (GAR)	*purD*	89
GAR transformylase	↓ formylglycinamide ribotide (FGAR)	?	
FGAM synthetase	↓ formylglycinamidine ribotide (FGAM)	*purG*	53
AIR synthetase	↓ 5-aminoimidazole ribotide (AIR)	*purI*	55
AIR carboxylase	↓ carboxyaminoimidazole ribotide (CAIR)	*purE*	12
SACAIR synthetase	↓ 5-aminoimidazole-4-(N-succinylo-carboxamide) ribotide (SACAIR)	*purC*	53
Adenylosuccinate lyase	↓ 5-aminoimidazole-4-carboxamide ribotide (AICAR)	*purB*	25
AICAR transformylase	↓ 5-formaminoimidazole-4-carboxamide ribotide (FAICAR) ↓ IMP	*purH*	89
Adenylosuccinate synthetase	IMP ↓ Adenylosuccinate	*purA*	94
Adenylosuccinate lyase	↓ AMP	*purB*	25
IMP dehydrogenase	IMP ↓ XMP	*guaB*	53
GMP synthetase (xanthylate aminase)	↓ GMP	*guaA*	53

[a]From Bachman, B. J., and Low, K. B. (1980) *Microbiol. Rev.* 44, 1.