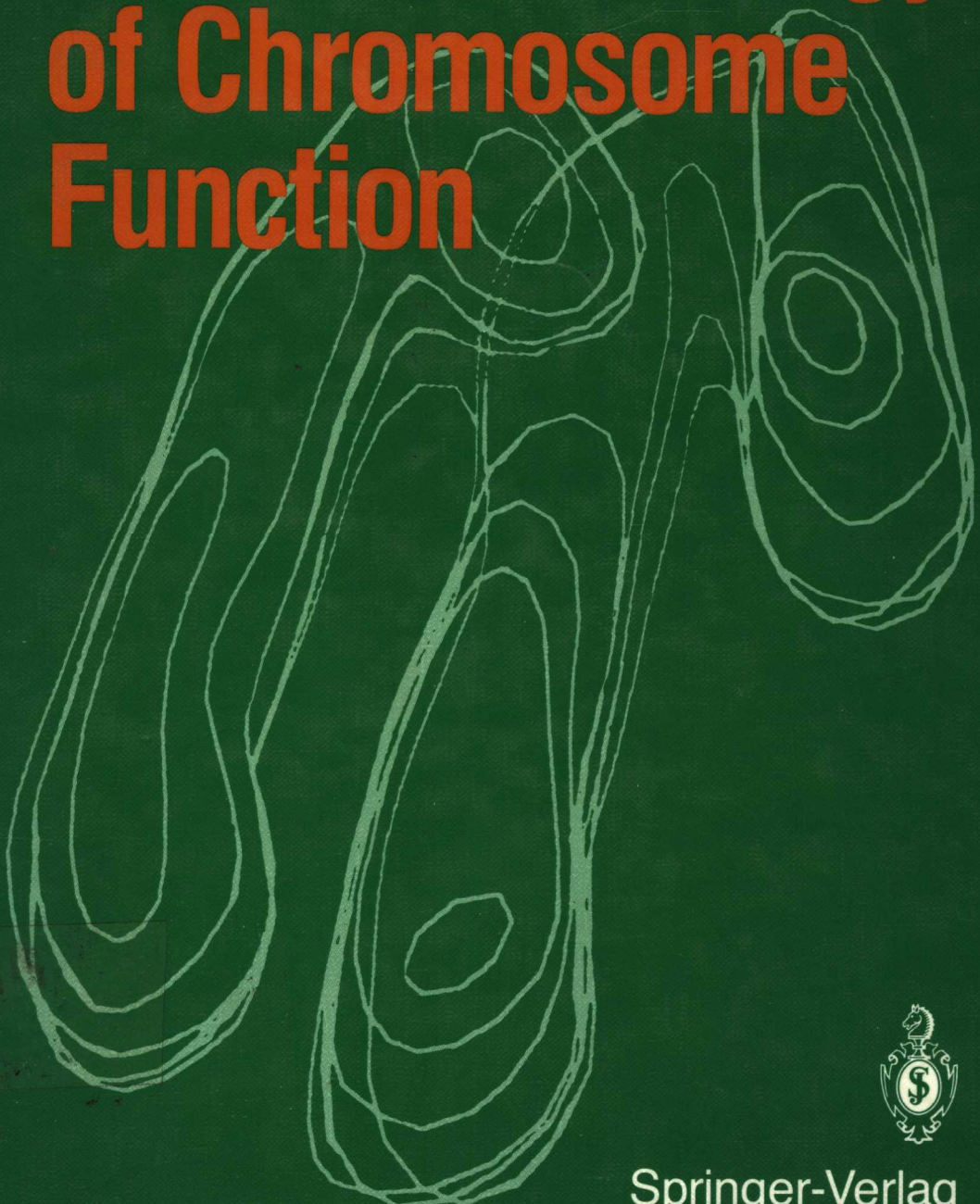
Kenneth W. Adolph

Editor

Molecular Biology of Chromosome Function



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General Introduction

Chromosomes have structure, determined by the interactions of proteins with DNA, and chromosomes have functions, in particular, replication of DNA and transcription of messenger RNA. Chromosome structure and function are not separate topics, since chromosome organization profoundly influences the activity of the genome in replication and transcription. This is especially clear for higher cells, including human cells, in which chromatin fibers are created by the binding of histone proteins to the DNA, and folding of the fibers produces mitotic chromosomes and interphase nuclei. The intricate organization of DNA in higher cells is now recognized as being closely involved with genome activity. Many fundamental results have originated from studies of bacterial and viral systems, which have been systems of choice because of their less complex life cycles. The processes of replication and transcription show differences between the higher and simpler systems (e.g., different enzymes and protein factors are involved). But the parallels are as striking as the differences in detail. Even for bacteria and viruses, a full understanding of these processes will require integrating the results of molecular biology with those of structural biology and cell biology.

Three important subjects are covered in this volume: DNA replication and recombination, gene transcription, and chromosome organization. The sections dealing with replication and transcription examine recent results obtained by applying the techniques of molecular biology and biochemistry. Eukaryotic, prokaryotic, and viral systems are discussed. The emphasis in the third section, which concerns chromosome organization, is on molecular cell biology. The information that is presented was derived from techniques of structural biology and biophysics, including computer graphics and X-ray crystallography, as well as biochemistry and cell biology. The book begins with chapters describing fundamental topics on DNA replication and recombination: DNA precursor synthesis, replication fork propagation, and the role of recA protein. Viral replication (SV40, HIV) is also covered. Chapters then follow that review basic aspects of the process of gene transcription: repressor proteins, RNA

polymerases, transcription termination, pre-mRNA splicing, ribonucleoproteins. The topics that conclude the book concern chromosome structure: the 3-D arrangement of human mitotic chromosomes, DNA binding proteins, histones, and protamines.

The book therefore provides a broad overview of centrally important subjects regarding the functions and structure of chromosomes. It is hoped that the work will prove to be of value for a variety of interested readers.

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Section I DNA Replication and Recombination

The two basic functions of the genomes of all living systems are replication and transcription. Important topics concerning the replication of DNA and RNA genomes are discussed in this section, while the transcription of messenger RNA from a DNA template is covered in the section that follows. For most organisms, including humans, the interaction of replication proteins with DNA is at the heart of the replication process. But the process is complicated by the organization of the DNA double helix into chromosomes which, in cells of higher organisms, consist of the DNA tightly bound to the histones and nonhistone proteins. The DNA-protein chromatin fibers are, in turn, arranged to form the characteristic structures of chromosomes. Understanding DNA replication in prokaryotes such as the bacterium *Escherichia coli* is complicated by the circular nature of the DNA molecule and by the fact that it is folded into a "nucleoid" in the intact cell. Viral chromosomes, even though tiny compared to those of bacteria and higher cells, also show distinctive features in their replication. This is true for the DNA-containing viruses such as the simian virus 40 (SV40) and for viruses with RNA genomes such as HIV, the human immunodeficiency virus.

Investigations of DNA replication are at an advanced level. The biochemistry of replication for a variety of systems is known in considerable detail. Not only has the enzymology of the DNA polymerases been extensively characterized, but precise roles have been revealed for a number of other important proteins associated with replication. The identification of many of the components involved in duplicating the DNA helix has demonstrated that the process is far from simple. The activity of DNA polymerase itself is but one event in a complex chain of events. The protein-DNA and protein-protein interactions at the replication fork must be considered, as must the roles of additional molecules that regulate DNA replication. These include the enzymes of DNA precursor synthesis.

Besides replication, DNA is involved in additional significant

processes. Homologous genetic recombination, discussed in this section, produces DNA rearrangements in the genetic transformation of prokaryotes, in DNA repair, and in other situations. The replication of certain viruses can be considered as model systems that illuminate general aspects of replication; SV40, for instance, contains a minichromosome with histones bound to the DNA to create nucleosomes. However, with retroviruses such as HIV, a genome of RNA is replicated and a DNA duplex is secondarily produced for integration into human chromosomes. Variety is clearly a key word in describing viral replication, as the chapters in this section illustrate.

Chapter 1
Enzymes of DNA Precursor Synthesis and the Control of DNA Replication

Christopher K. Mathews

Although the enzymes of DNA precursor biosynthesis were originally described in the 1950s and 1960s, it is still timely to discuss these proteins with respect to their involvement in regulating DNA replication. This chapter will focus on current research that emphasizes the genetics and cell biology of these enzymes: How do the enzymes interact in cells, with each other, and with replication proteins? What controls reaction fluxes through individual enzymes in vivo? How do these fluxes vary through the cell cycle, and what factors control the variations? What are the effective concentrations of deoxyribonucleoside triphosphates (dNTPs*) at replication sites? How do variations in dNTP pools affect the rate and accuracy of DNA replication? What are the metabolic and genetic consequences of inhibiting a particular enzyme with an antimetabolite? Metabolic inhibitors provide useful probes for understanding compartmentation and control. In turn, this understanding could lead to the more effective use of antimetabolites as anticancer, antimicrobial, or antiviral agents.

Although direct roles of dNTPs in regulating DNA replication have not been established, the synthesis of DNA precursors is closely coordinated with DNA replication in most organisms studied. Such coordination might be expected a priori, simply from the facts that DNA replication is localized both in time and in space within a cell and that replication uses precursors that have few if any additional metabolic roles. This chapter will focus on mechanisms involved in this coordination and on the extent to which dNTPs are involved in controlling the rate and fidelity of DNA replication.

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* Abbreviations: rNMP, rNDP, rNTP, ribonucleoside mono-, di-, and triphosphate, respectively; dNMP, dNDP, dNTP, deoxyribonucleoside mono-, di-, and triphosphate, respectively.

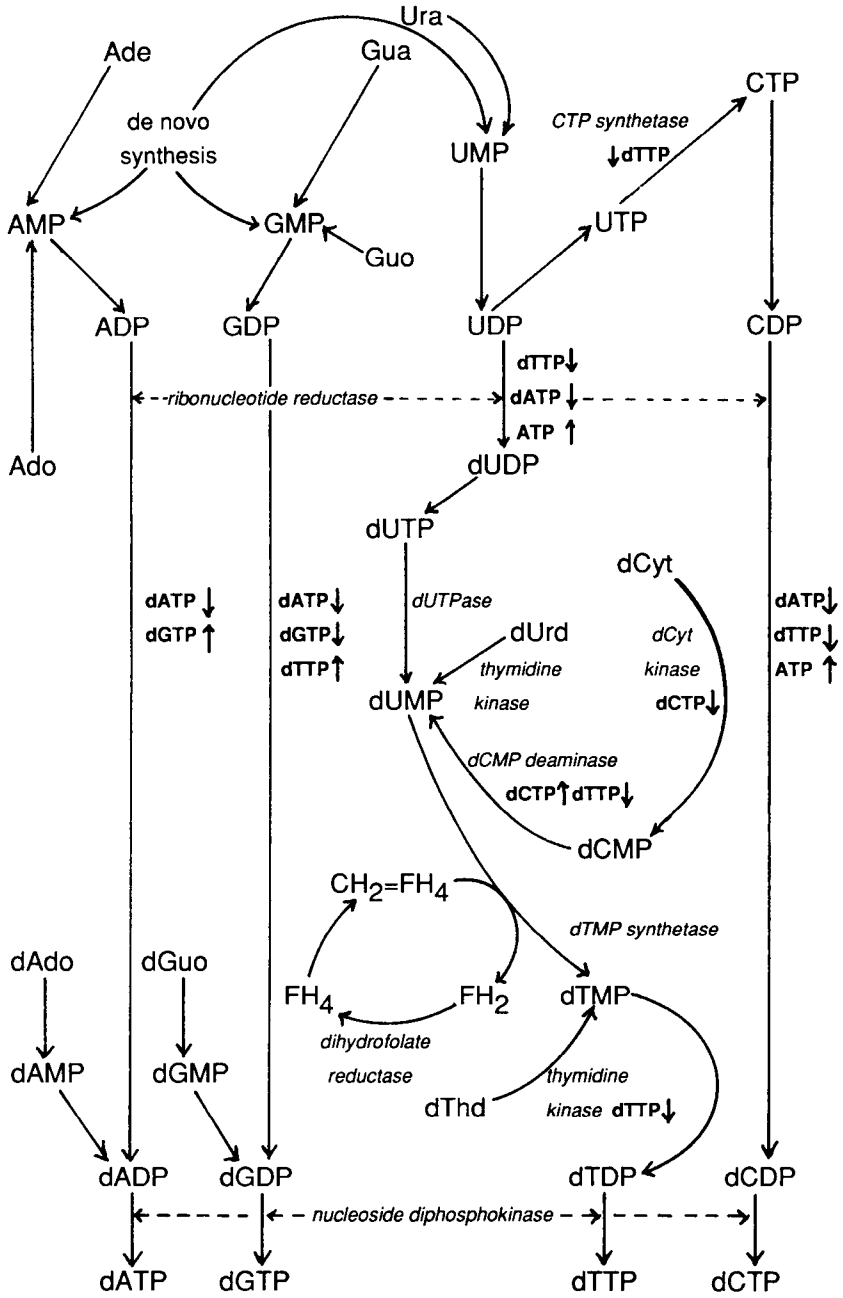
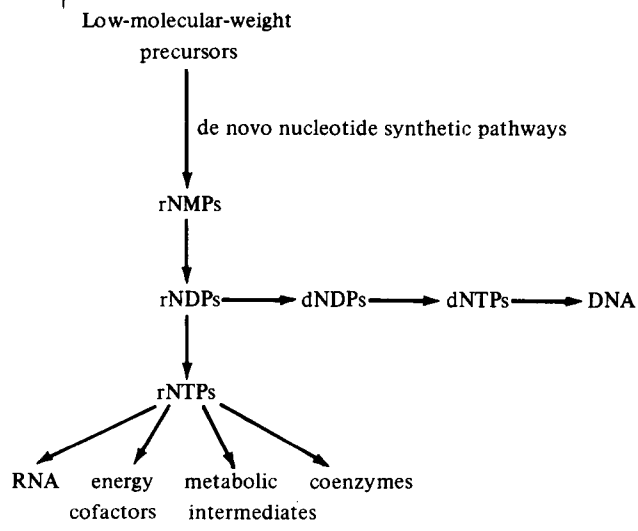


Figure 1.1. Pathways of deoxyribonucleoside triphosphate biosynthesis in mammalian cells. Major enzymes and allosteric effectors are identified. Arrows indicate whether each allosteric effect is positive or negative.

Pathways of dNTP Biosynthesis and Regulation of the Enzymes

Figure 1.1 summarizes the enzymatic pathways of dNTP biosynthesis in mammalian cells and identifies effectors for reactions known to be controlled in allosteric fashion. Some variations are found in microbial systems and in virus-infected cells, and a few of these variations will be noted. The enzymatic reactions in these pathways are well described in most biochemistry textbooks. A particularly useful review is provided in Chapter 1 of Kornberg's excellent book [1].

The pathways in Figure 1 can be represented more simply as follows:



In most cells RNA is 5 to 10 times more abundant than DNA. Because of this and the numerous metabolic roles played by ribonucleotides, only a small percentage of the carbon flux through the de novo purine and pyrimidine synthetic pathways is diverted toward deoxyribonucleotides. Great interest has been paid to ribonucleotide reductase, because it catalyzes the first committed step in DNA replication. Additional interest derives from the fact that the reaction—reduction of a nucleotide ribose moiety to deoxyribose—is mechanistically unusual. Early attention focused also on the enzyme that generates the other distinctive functional group in DNA—i.e., thymidylate synthase—which forms the methyl group of thymine nucleotides. Both ribonucleotide reductase and thymidylate synthase displayed low activity when assayed in extracts during

early investigations, and it was suggested that either or both enzymes are rate limiting for DNA replication.

Although our understanding of the control of DNA replication is far from complete, developments in the 1970s and 1980s have indicated that the major regulated step is initiation from replicon origins, with replicative chain elongation being a secondary target. Therefore, the simple idea that thymidylate synthase or ribonucleotide reductase could be rate limiting for DNA replication has been abandoned. However, ribonucleotide reductase does play an important regulatory role of a more complex nature, through the fact that this one enzyme system participates in the synthesis of all four DNA precursors. Through interactions of nucleoside triphosphates with two distinct allosteric sites on the enzyme molecule, the relative abilities of the enzyme to reduce ADP, CDP, UDP, and GDP are modulated so that the cell produces dNTPs in the correct relative proportions needed for DNA replication [reviewed in 2]. The biological importance of these mechanisms was underscored in a series of important papers from Martin's laboratory [cf. 3]. These investigations showed that mutations altering the control sites of mouse ribonucleotide reductase led to dNTP pool imbalances and genetic abnormalities, including elevated spontaneous mutation rates.

Two other mechanisms control ribonucleotide reductase. First, the enzyme contains a tyrosine free radical that plays an essential role in catalysis. Reichard's laboratory has characterized an enzyme system in *E. coli* that generates the radical and that may control the proportion of enzyme in the active and inactive forms [reviewed in 4], although the nature of the control has not yet been elucidated. Second, the level of the enzyme protein is closely coordinated with the proliferative state of the cell. In synchronized mammalian cells the amount of enzyme protein increases at least 10-fold as cells enter S phase. The availability of cDNA probes has led to a demonstration that posttranscriptional mechanisms are involved in this regulation [5].

By contrast, thymidylate synthase is not considered to be an important regulatory target. No allosteric effectors are known, and it seems likely that flux through this reaction is determined by intracellular concentrations of substrates and products. However, two other enzymes of dNTP biosynthesis are, like ribonucleotide reductase, regulatory targets that help to maintain appropriate dNTP pool sizes. Deoxycytidylate deaminase is a branch point between dCTP and dTTP biosynthesis. Mutations that affect the control of this enzyme by dCTP and dTTP (positive and negative, respectively) alter the balance of dCTP and dTTP pools and similarly effect spontaneous mutation rates [6,7]. The other enzyme, CTP synthetase, is involved in ribonucleotide biosynthesis. However, mutations affecting the feedback inhibition of this enzyme by dTTP have a complex phenotype that includes both elevated cytidine and deoxycytidine nucleotide pools and increased spontaneous mutation rates [8,9].

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