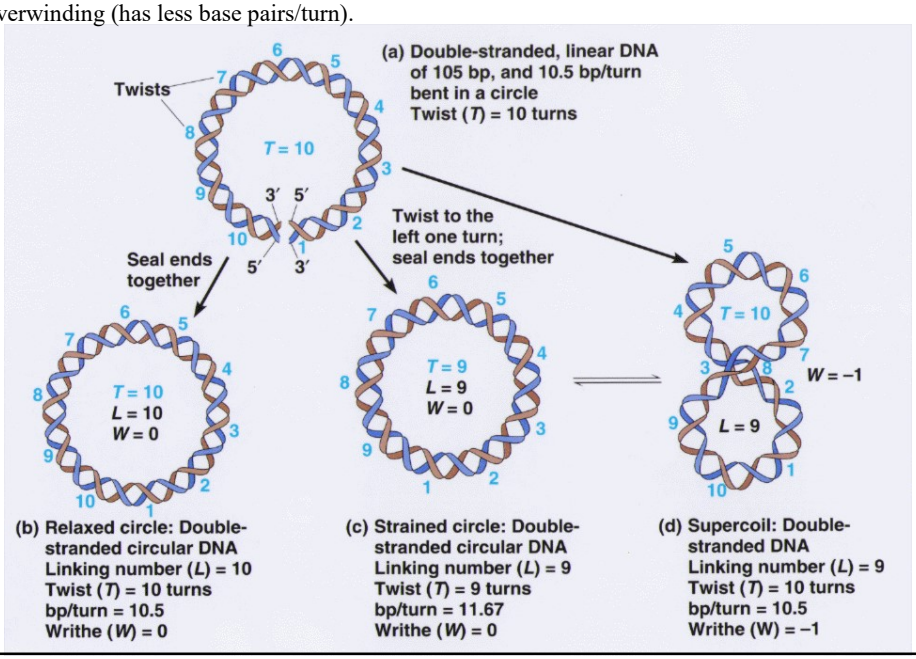
Supercoiled DNA

Negative supercoiling is underwinding (has more base pairs/turn). Positive supercoiling is overwinding (has less base pairs/turn).



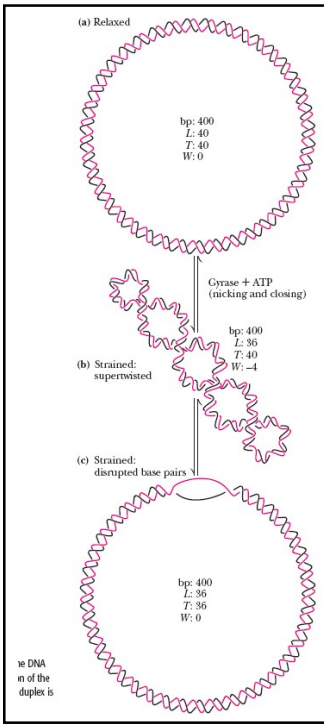
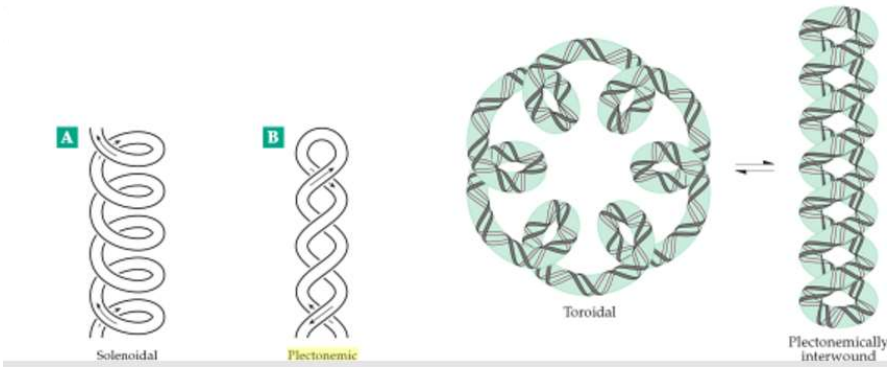


FIGURE 11.26 A 400-bp circular DNA molecule in different topological states: (a) relaxed, (b) negative supercoils distributed over the entire length, and (c) negative supercoils creating a localized single-stranded region.



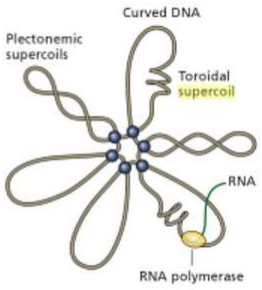


Figure 4.17 Supercoiled DNA domains in the *E. coli* chromosome. The drawing shows the presence of both plectonemic loops and loops containing toroidal or solenoidal structures; the proteins involved are not depicted. This is a simplified cartoon of the chromosome; in real life, there are perhaps as many as 400 different domains. The loops exist due to the presence of specific structural proteins bound at the bases of the loops. Intrinsically curved DNA that bends spontaneously because of a specific sequences of bases tends to be localized at the tips of supercoils. (Adapted from Willenbrock H & Ussery DW [2004] *Genome Biol* 5:252–256. With permission from BioMed Central.)

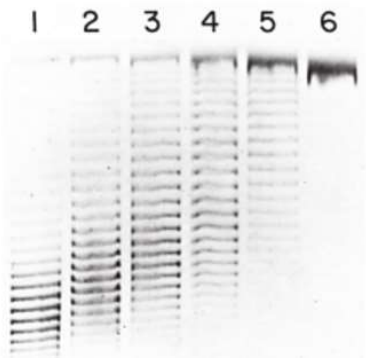


Figure 5-20 Electrophoresis of DNA from the SV40 virus with varying numbers of superhelical turns. Molecules of the native DNA (lane 1) move rapidly toward the anode as a series of bands, each differing from its neighbors by one superhelical turn. The average number of superhelical turns is about 25. Incubation with a topoisomerase from human cells causes a stepwise removal of the superhelical turns by a cutting and resealing of one DNA strand. The DNA incubated with this enzyme at 0°C for periods of 1, 3, 6, 10, and 30 min (lanes 2–6) is gradually converted to a form with an average of zero supercoils. For details see Keller.[202,212] Electrophoresis was carried out in an 0.5% agarose–1.9% polyacrylamide slab gel (17 x 18 x 0.3 cm). The bands of DNA were visualized by staining with the fluorescent intercalating dye ethidium bromide. Photograph courtesy of Walter Keller.

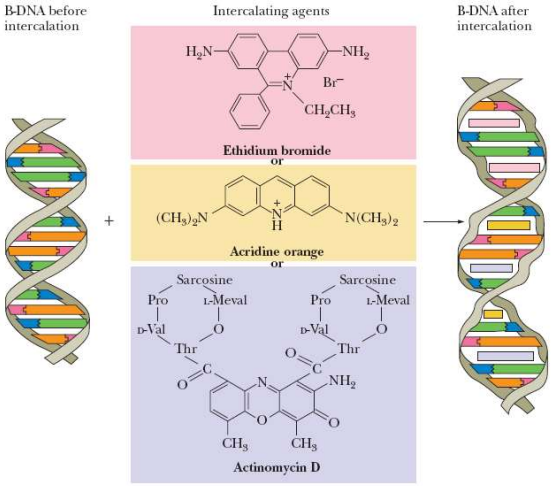


FIGURE 11.14 The structures of ethidium bromide, acridine orange, and actinomycin D, three intercalating agents, and their effects on DNA structure.

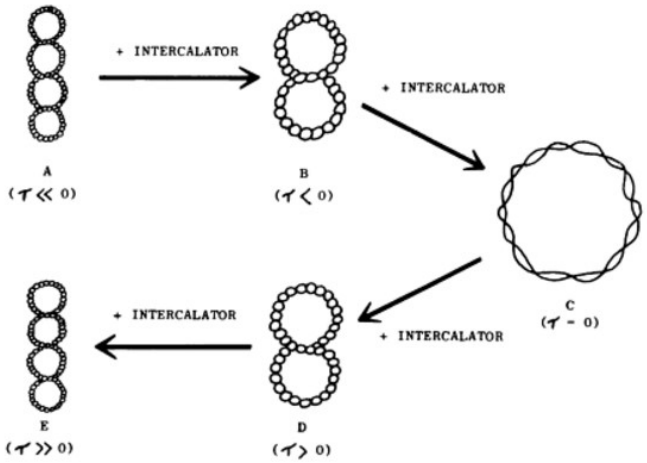


Fig. 10. Diagrammatic representation of the effects of intercalating agents on superhelical covalently closed circular DNA. A, naturally isolated supercoiled ccc-DNA (negative supercoiled); B, partially relaxed ccc-DNA; C, completely relaxed ccc-DNA; D, partially reverse coiled ccc-DNA; E, completely reverse-coiled ccc-DNA (positively supercoiled). τ represents the superhelix density of a given species of ccc-DNA, which is a measure of the extent of supercoiling of the DNA duplex.

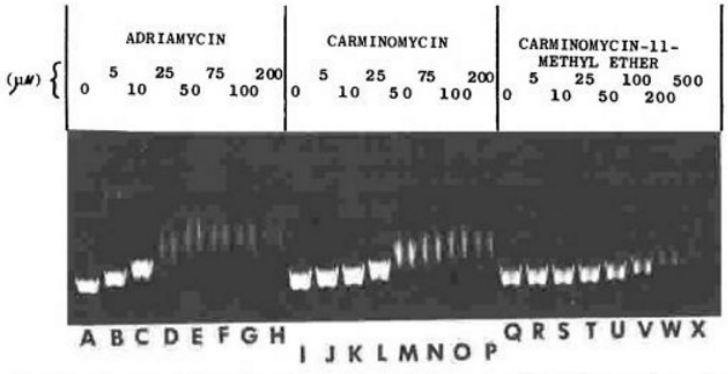


Fig. 11. Agarose gel electrophoretic separations of anthracycline–PM-2 DNA reaction products. Reactions were performed and agarose gel electrophoresis conducted as previously reported (DuVernay *et al.*, 1980). Direction of electrophoresis is from top to bottom, with the fastest-migrating band being the superhelical ccc–PM-2 DNA and the slowest-migrating band (faintly visible) being the relaxed form DNA. Lanes A through H correspond to increasing concentrations of adriamycin of 0, 5, 10, 25, 50, 75, 100, and 200 μM, respectively. Lanes I through P correspond to identical concentrations of carminomycin. Lanes Q through X correspond to increasing concentrations of carminomycin-11-methyl ether of 0, 5, 10, 25, 50, 100, 200, and 500 μM, respectively. Electrophoresis was at 5 V/cm for 10 hr at room temperature. Gels were stained with 0.5 μg/ml of ethidium bromide.

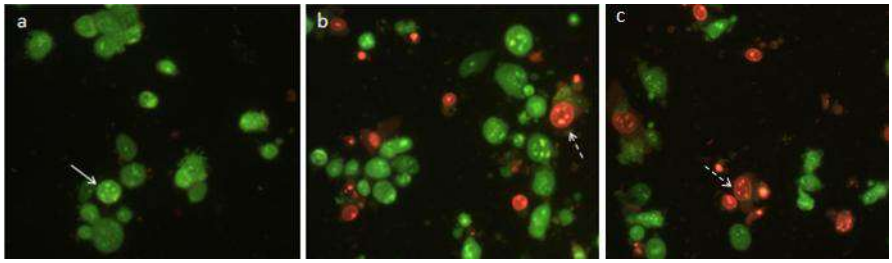
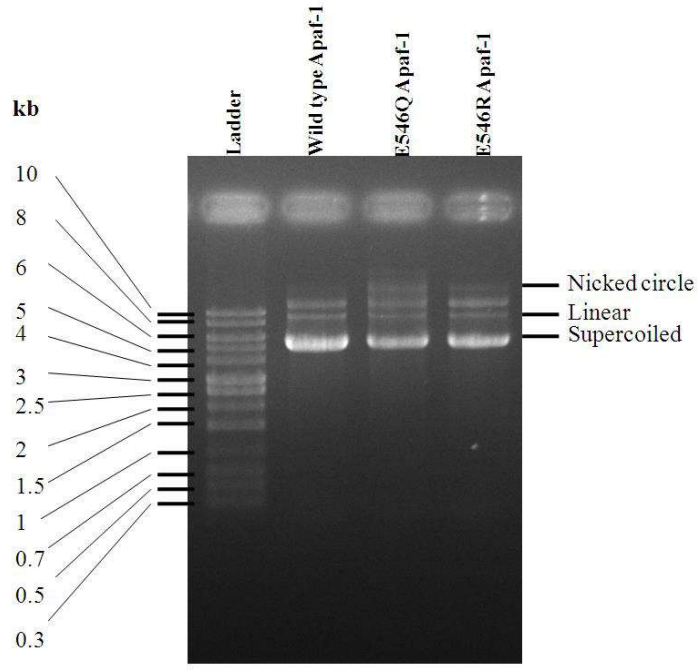


Figure 5. Acridine orange/ethidium bromide fluorescent staining of MDA-MB-231 cells for determine apoptosis: (a) DMSO 1% as control; (b) cells treated with IC_{50} concentration of compound **4a** (c) cells treated with IC_{50} concentration of etoposide as positive control for 24 hr. White arrow indicates live cells and dashed arrow indicates apoptotic cells. The images of cells were taken with a fluorescence microscope at 400×



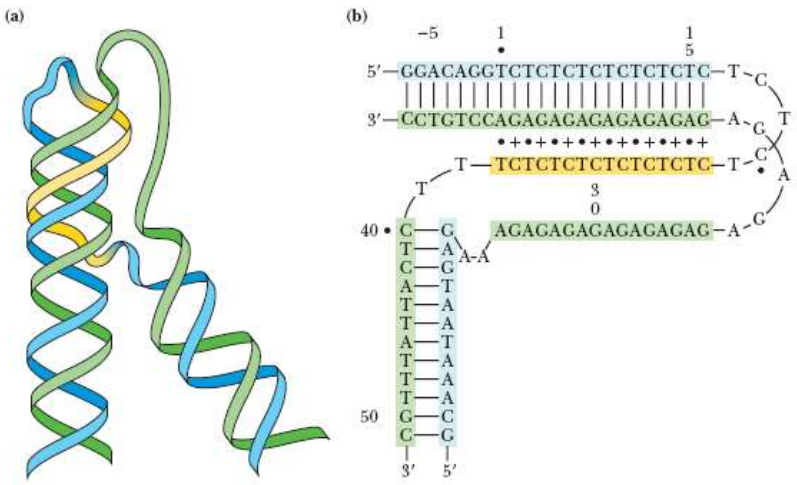


FIGURE 11.18 H-DNA. (a) The pyrimidine-rich strands of the duplex regions are blue, and the purine-rich strands are green. The Hoogsteen base-paired pyrimidine-rich strand in the triplex (H-DNA) structure is yellow. (b) Nucleotide sequence representation of H-DNA formation. T:A Hoogsteen base pairing leading to triplex formation is shown by dots; C^+-G Hoogsteen base pairing leading to triplex formation is shown by + signs. (Adapted from Htun, H., and Dahlberg, J. E., 1989. Topology and formation of triple-stranded H-DNA. *Science* **243**:1571–1576.)

Hoogsteen Base Pairs and DNA Multiplexes The A:T and G:C base pairs first seen by Watson (Figure 11.8) are the canonical building blocks for DNA structures. However, Karst Hoogsteen found that adenine and thymine do not pair in this way when crystallized from aqueous solution. Instead, they form two H bonds in a different arrangement (Figure 11.16). Further, Hoogsteen observed that, in mildly acidic solutions, guanine and cytosine form base pairs different from Watson–Crick G:C base pairs.

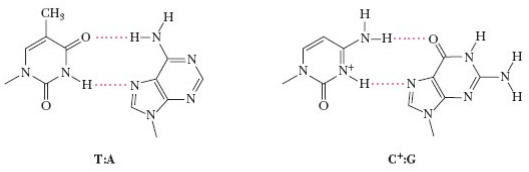


FIGURE 11.16 Hoogsteen base pairs: A:T *(left)* and C^+:G *(right)*.

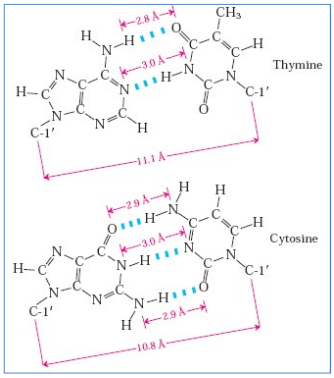


FIGURE 8–11 **Hydrogen-bonding patterns in the base pairs defined by Watson and Crick.** Here as elsewhere, hydrogen bonds are represented by three blue lines.

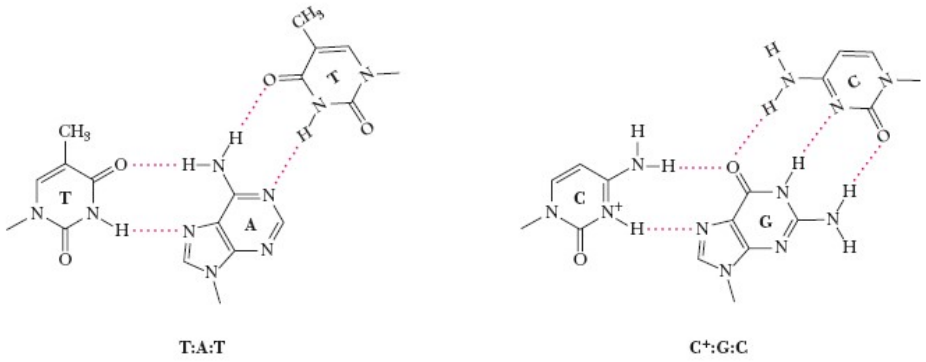
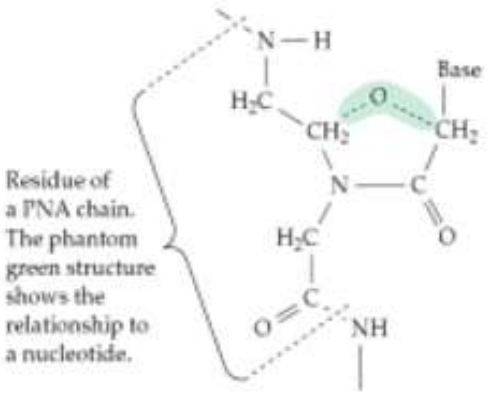
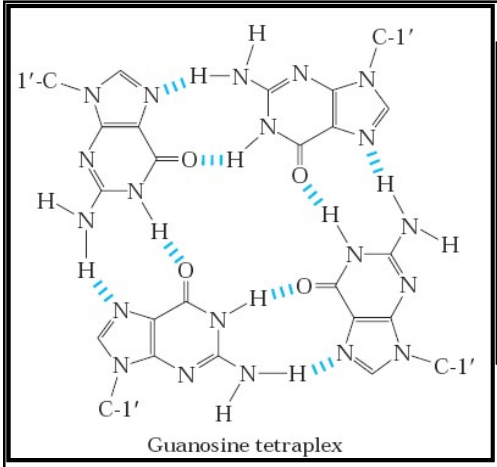
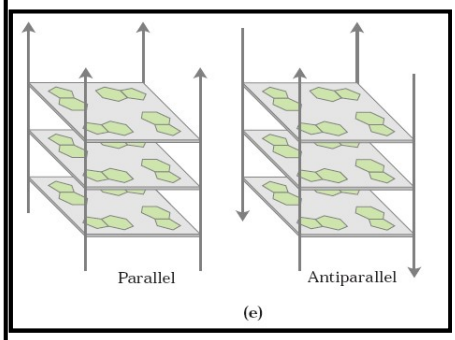


FIGURE 11.17 Base triplets formed when a purine interacts with one pyrimidine by Hoogsteen base pairing and another by Watson–Crick base pairing.

These Hoogsteen base pairs depend upon protonation of cytosine N-3 (Figure 11.16) and have only two H bonds, not three. In both A:T and G:C Hoogsteen base pairs, the purine N-7 atom is an H-bond acceptor. The functional groups of adenine and guanine that participate in Watson–Crick H bonds remain accessible in Hoogsteen base pairs. Thus, base triplets can form, as shown in Figure 11.17, giving rise to TAT and a C^+GC triplets, where each purine interacts with one of its pyrimidine partners through Hoogsteen base pairing and the other through Watson–Crick base pairing.

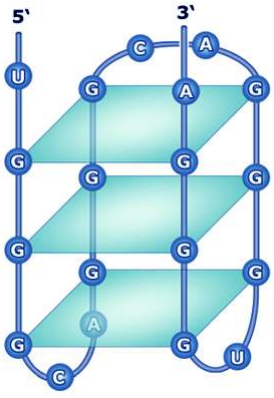


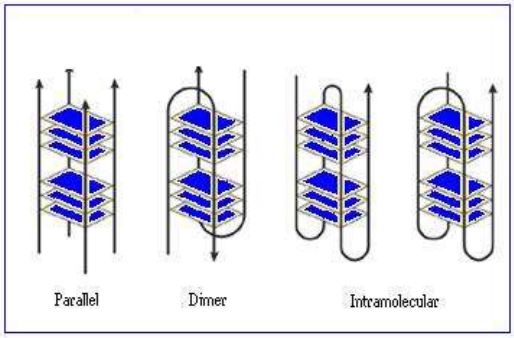




Four DNA strands can also pair to form a tetraplex (quadruplex), but this occurs readily only for DNA sequences with a very high proportion of guanosine residues.

Quadruplexes can be formed from one, two or four separate strands of DNA (or RNA) and can display a wide variety of topologies.

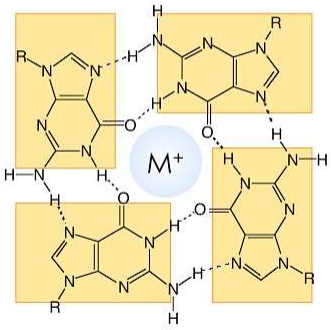




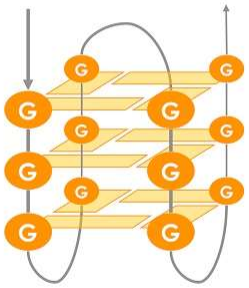
G-quartets are stabilized by a monovalent cation (Na+ or K+) localized in the center of the structure

Telomeric DNA

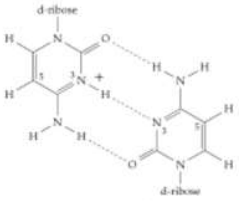
5′–...TTAGGGTTAGGGTTAGGGTTAGGG...–3′



G-quartet



G-quadruplex (G4)



Hemiprotonated d(CH+•C)

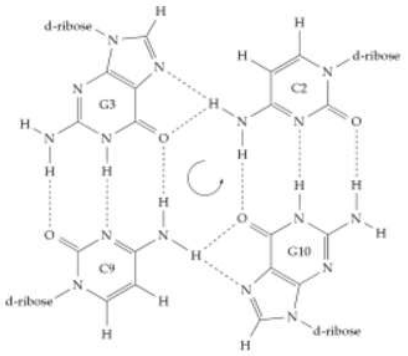
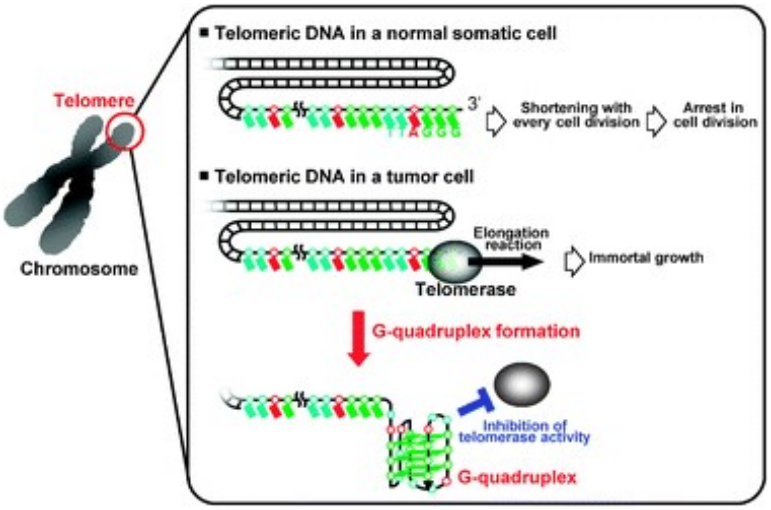


Figure 5-26 Structure of a G•C+G•C+ tetrad present in a quadruplex structure formed by the oligonucleotide d(GCG-CTTTGCGC) in Na^+-containing solution. See Kettani *et al.*[276]



Bioinformatics and molecular sequence analysis indicates that G-quadruplexes are over-represented in specific regions of the genome with key biological contexts. This includes DNA telomere ends and promoter regions (translation start sites) of several important oncogenes [21,33,38,39]. It has been shown that the formation of quadruplexes inhibits the telomere extension by the telomerase enzyme, which is up-regulated in cancer cells, as well as negatively regulating oncogene's transcription [40,41] Because of its biological significance and antitumor potential, the G-quadruplex has attracted intense interest as an important target for drug design and development and there is a huge interest in design and development of small molecules to target these structures. A large number of so-called G-quadruplex ligands, displaying varying degrees of affinity and more importantly selectivity, have been reported [42,43].

Table 4. Sequences in cancer-related genes that have been identified as forming quadruplex structures

Gene	Sequence	Ref
c-myc	*Pu27*	88
	Myc-2345	88
	Myc-1245	
	Myc-22	89
	Pu24I	90
c-kit	*ckit1*	91
	ckit2	92
VEGF		96
HIF-1α		95
bcl-2		93
k-ras		97
Rb		98

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doi:10.1093/nar/gkl655

SURVEY AND SUMMARY

Quadruplex DNA: sequence, topology and structure

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ABSTRACT

G-quadruplexes are higher-order DNA and RNA structures formed from G-rich sequences that are that short G-rich sequences at the ends of telomeric DNA in eukaryotic chromosomes can associate together in physiological ionic conditions to form discrete four-stranded structures (variously termed quadruplexes, tetraplexes or G4