8.3: A Case Study- Tris(phenanthroline) Metal Complexes

Now we may examine in detail the interaction of one class of metal complexes with nucleic acids, how these complexes bind to polynucleotides, the techniques used to explore these binding interactions, and various applications of the complexes to probe biological structure and function. Tris(phenanthroline) metal complexes represent quite simple, well-defined examples of coordination complexes that associate with nucleic acids. Their examination should offer a useful illustration of the range of binding modes, reactivity, techniques for study, and applications that are currently being exploited and explored. In addition, we may contrast these interactions with those of other transition-metal complexes, both derivatives of the tris(phenanthroline) family and also some complexes that differ substantially in structure or reactivity.

Binding Interactions with DNA

Tris(phenanthroline) complexes of ruthenium(II), cobalt(III), and rhodium(III) are octahedral, substitutionally inert complexes, and as a result of this coordinative saturation the complexes bind to double-helical DNA through a mixture of noncovalent interactions. Tris(phenanthroline) metal complexes bind to the double helix both by intercalation in the major groove and through hydrophobic association in the minor groove.[11b,40] Intercalation and minor groove-binding are, in fact, the two most common modes of noncovalent association of small molecules with nucleic acids. In addition, as with other small molecules, a nonspecific electrostatic interaction between the cationic complexes and the DNA polyanion serves to stabilize association. Overall binding of the tris(phenanthroline) complexes to DNA is moderate (log K = 4).[41]

The extent of intercalative versus groove binding is seen to depend upon environmental conditions, such as temperature and ionic strength, the charge of the metal center, and the DNA base sequence; groove binding is favored at AT-rich sequences.[41] Second-generation mixed-ligand derivatives of the tris(phenanthroline) series have been prepared, and their interactions with DNA have provided useful insight into the factors important for promoting either intercalation or groove binding.[42] Aromatic heterocyclic ligands with increased surface areas that are planar bind DNA with increasing avidity through intercalation, irrespective of the charge on the metal center. Intercalative binding constants greater than 10^7 M^{-1} can be easily achieved with planar heterocyclic ligands that jut out from the metal center. Not surprisingly, complexes containing ligands of increasing hydrophobicity that are not planar favor minor-groove binding.[28]

Critically important as well in determining the binding mode is the chirality of the metal complex.[40] Intercalation into the right-handed helix favors the Δ-isomer, whereas groove binding favors the Λ-isomer. Figure 8.7 illustrates these symmetry-selective interactions. In intercalation, we consider that one phenanthroline inserts and stacks in between the base pairs, essentially perpendicular to the helix axis. For the Δ-isomer, once intercalated, the ancillary non-intercalated ligands are aligned along the right-handed groove of the helix. For the Λ-isomer, in contrast, with one ligand intercalated, the ancillary ligands are aligned in opposition to the right-handed groove, and steric interactions become evident between the phenanthroline hydrogen atoms and the phosphate oxygen atoms. Increasing the steric bulk on these phenanthrolines furthermore increases the enantioselective preference for intercalation of the Δ-isomer.[40,43] *For intercalation*, then, *the chiral discrimination depends on matching the symmetry of the metal complex to that of the DNA helix. For groove binding*, where the metal complex is thought to bind *against* the helix, instead *it is a complementary symmetry that is required*. In our model for groove binding of the tris(phenanthroline) metal complex, two phenanthroline ligands are likely bound against the right-handed helical groove, stabilized through hydrophobic association. For the Λ-isomer, bound in this fashion, the ligands lie against and complement the right-handed groove; with the Δ-isomer, the ligands oppose the groove, and no close surface contacts are made.

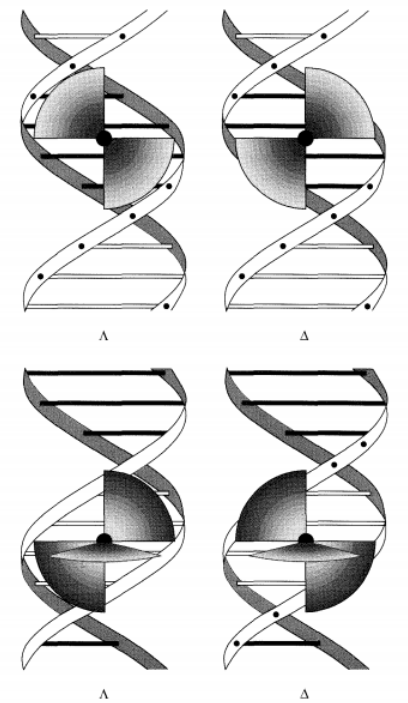


Figure 8.7 - Enantiomeric discrimination in binding to DNA. Shown above is the basis for the preference for Δ-Ru(phen)$_3^{2+}$ upon intercalation and Λ-Ru(phen)$_3^{2+}$ for surface binding against a right-handed helix. With intercalation (top) the symmetry of the metal complex matches the symmetry of the helix; steric interactions preclude a close association of the Λ-isomer. With groove binding (bottom), where the metal complex binds against the minor-groove helical surface, complementary symmetries are required, and it is the Λ-isomer that is preferred.

Intercalation of metal complexes in DNA is not uncommon. Lippard and coworkers first established metallointercalation by Pt(II) complexes in the 1970s.[18,20] Square-planar platinum(II) complexes containing the terpyridyl ligand were shown to intercalate into DNA. In an elegant series of x-ray diffraction experiments on DNA fibers, Lippard illustrated the requirement for planarity in the complex.[18,44] Although (phen)Pt(en)$^{2+}$ and (bpy)pt(en)$^{2+}$ were shown to intercalate into the helix, (pyr)$_2$Pt(en)$^{2+}$, with pyridine ligands rotated out of the coordination plane, could not. Complex planarity is in itself insufficient to promote intercalation, however. *Cis*-(NH$_3$)$_2$PtCl$_2$ or even *cis*-(NH$_3$)Pt(en)$^{2+}$ does not appear to intercalate into a helix, despite full planarity. Instead, aromatic heterocyclic ligands must be included in order to promote dipole-dipole interactions with the heterocyclic bases stacked in the helix. Indeed, planarity of the full complex is not required. Intercalation is not restricted to coordination complexes that are square planar. The tris(phenanthroline) complexes represented the first examples of "three-dimensional intercalators" and illustrated that octahedral metal complexes could also intercalate into the helix.[40,45,46] Here one can consider the partial intercalation of one ligand into the helix, providing the remaining ligands on the complex an opportunity to enhance specificity or reactivity at a given site.

Curiously, one unique and apparently general characteristic of metallointercalators is their preference for intercalation from the *major groove* of the helix. Most small molecules associate with DNA from the minor groove, but metallointercalators, both those that are square planar, such as (terpyridyl)platinum(II) complexes, and those that are octahedral, such as the tris(phenanthroline) metal complexes, appear to intercalate into the major groove. This then mimics quite well the association of much larger DNA-binding proteins with the helix; DNA regulatory proteins generally appear to target the major groove. The reason why metallointercalators favor major groove association is still unclear.

Transition-metal complexes with aromatic ligands also generally associate by minor-groove binding or through the mix of intercalative and groove-bound interactions. Cu(phen)$_2^+$, a tetrahedral complex, appears to favor minor-groove binding over intercalation.[26] Perhaps the tetrahedral coordination does not permit appreciable overlap of the phenanthroline ring with the bases in an intercalative mode. Metalloporphyrins, despite their large expanse and the presence commonly of nonplanar substituents, appear to bind to double-helical DNA both by intercalation and by minor-groove binding at AT-rich sequences.[47] Occupation of the porphyrins by transition-metal ions, such as Cu(II), which bind axial ligands, leads to the favoring of groove binding over intercalation. Figure 8.8 illustrates some of the complexes that bind DNA noncovalently.

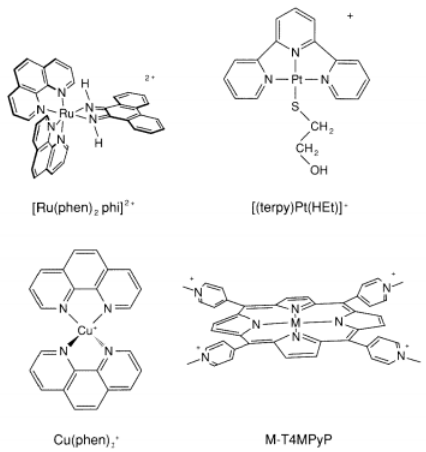


Figure 8.8 - Some metal complexes that bind DNA noncovalently primarily through intercalation (top) or binding in the minor groove (bottom). Some metalloporphyrins also primarily associate via intercalation.

The tris(phenanthroline) metal complexes themselves do not offer an illustration of hydrogen-bonding interactions with the helix, since these ligands lack hydrogen-bonding donors and acceptors, but as mentioned already, hydrogen bonding of coordinated ligands to the helix can add some measure of stabilization, comparable to, but likely no greater in magnitude than, that provided by intercalative stacking, hydrophobic, or dispersive interactions. Indeed, mixed-ligand derivatives of the phenanthroline complexes have been prepared that include hydrogen-bonding groups (amides, hydroxyls, and nitro substituents) on the ancillary phenanthroline ligands, and these have shown no greater avidity for double-helical DNA than their counterparts with hydrophobic substituents.[42] A large number of weak hydrogen-bonding interactions to DNA by one complex can be stabilizing, however, as with, for example, hexaamminecobalt(III) or hexaaquoterbium(III).

Tris(phenanthroline) metal complexes also do not offer an opportunity to explore covalent binding interactions with the helix in greater detail, but these interactions are, in fact, a major focus of Chapter 9, concerned with the mode of action of cisplatin. One derivative of the tris(phenanthroline) series, $Ru(phen)_2Cl_2$, has been shown to bind to DNA covalently.[48] In aqueous solution the dichlororuthenium(II) complex undergoes hydrolysis to form an equilibrium mixture of bis(phenanthroline) diaquo and chloroaquo species. These species bind covalently to DNA, with preferential reactivity at guanine sites. It is interesting that the same structural deformations in the DNA evident upon binding *cis*-diammineplatinum units become apparent upon coordination of bis(phenanthroline)ruthenium(II). It is also noteworthy that the chiral preference in coordination is for the Λ-isomer. As with groove binding, direct coordination to base positions requires a complementary symmetry, with the the Λ-isomer binding *against* the right-handed groove. This preference for the Λ-isomer reaffirms that, rather than noncovalent intercalation (which would favor the Δ-isomer), covalent binding dominates the interaction. The energetic stabilization in direct coordination of the ruthenium(II) center is certainly more substantial than the weaker stabilization derived from intercalation. $Rh(phen)_2Cl_2^+$ and its derivatives have also been shown to bind covalently to DNA but only upon photoactivation, since light is needed to promote dissociation of the coordinated chloride and substitution of the nucleic acid base as a ligand.[49]

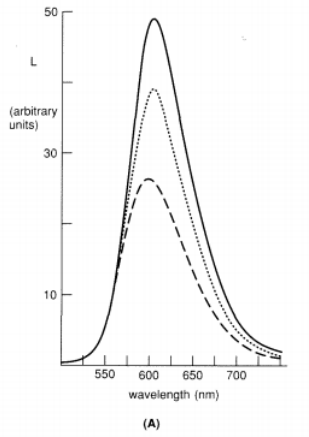
Techniques to Monitor Binding

Many of the same techniques employed in studying the basic chemistry of coordination complexes can be be used in following the binding of transition-metal complexes to nucleic acids, but biochemical methods, with their often exquisite sensitivity, become valuable aids as well in delineating specific binding interactions. Tris(phenanthroline) metal complexes are particularly useful to illustrate this point, since here the metal center in the complex is selected in terms of the technique used for examination.

Coordination complexes are often visibly colored, and these colorations provide a useful and sensitive spectroscopic handle in following fundamental reactions. This notion holds as well with tris(phenanthroline) metal complexes in their interactions with nucleic acids. $Ru(phen)_3^{2+}$ and its derivatives are highly colored because of an intense metal-to-ligand charge-transfer band (λ_{max} = 447 nm, $\epsilon = 1.9 \times 10^4\ M^{-1}cm^{-1}$). Furthermore, the complexes are highly photoluminescent (λ_{cm} = 610 nm, τ = 0.6 μs in aerated aqueous solution). On binding to nucleic acids these transitions are perturbed. Hypochromism is observed in the charge-transfer band, and intercalation leads to an increase in lifetime of the charge-transfer excited state.[43,46] Indeed, single-photon counting experiments show a biexponential decay in emission from $Ru(phen)_3^{2+}$ bound to double-helical DNA. The longer-lived component (τ = 2 μs) has been assigned as the intercalated component and the shorter-lived 0.6 μs component has been attributed to a mixture

of free and groove-bound species. These spectroscopic perturbations permit one to define equilibrium-binding affinities for the different components of the interaction as a function of metal-center chirality and under different solution conditions.[41] One can also follow the polarization of emitted light from the complexes after excitation with polarized light, and these studies have been helpful in describing the dynamics of association of the complexes on the helix.[41,43] Mixed-ligand complexes of ruthenium(II) show similar spectroscopic perturbations, and these have been used to characterize binding affinities and chiral preferences, as well as the extent of intercalation versus groove binding as a function of ligand substitution on the metal center.[42] The spectroscopic handle of the metal center therefore affords a range of experiments to monitor and characterize the binding of the metal complexes to polynucleotides.

Binding interactions of metal complexes with oligonucleotides can also be followed by NMR, and here as well the metal center offers some useful characteristics to exploit. As with organic DNA-binding molecules, shifts in the ^{1}H-NMR resonances of both the DNA-binding molecule and the oligonucleotide become apparent as a function of increased association with the helix. These shift variations can be used empirically to watch the dynamics of association and to gain some structural insights into the binding modes of the complexes on the helix. These kinds of experiments have been performed with tris(phenanthroline) complexes of ruthenium(II) and rhodium(III), where it was observed that the double-helical oligonucleotide is an exceedingly good chiralshift reagent to separate resonances in an enantiomeric mixture of the tris(phenanthroline) complexes.[50] For covalent binding molecules, such as *cis*-diammineplatinum(II), furthermore, the lowering of the pK_a of purine positions and therefore shifting of resonances as a function of coordination to an alternate site has been helpful as well in assigning the sites of covalent binding on the oligonucleotide.[51] But also in NMR experiments, special advantage can be taken of the metal center. For tris(phenanthroline) metal complexes, ^{1}H-NMR experiments[52] were performed on the paramagnetic analogues, $Ni(phen)_3^{2+}$ and $Cr(phen)_3^{3+}$. It was reasonable to assume the binding characteristics would be identical with their respective diamagnetic analogues, Ru(II) and Rh(III); yet paramagnetic broadening by the metal complexes of nearby resonances on the oligonucleotide would allow one to deduce where along the helix the complexes associate. Using this method the groove-binding interaction of the complexes was identified as occurring in the minor groove of the helix. Figure 8.9 illustrates the monitoring of DNA binding by tris(phenanthroline) metal complexes using both the luminescence characteristics of ruthenium(II) complexes and the paramagnetic characteristics of nickel(II).



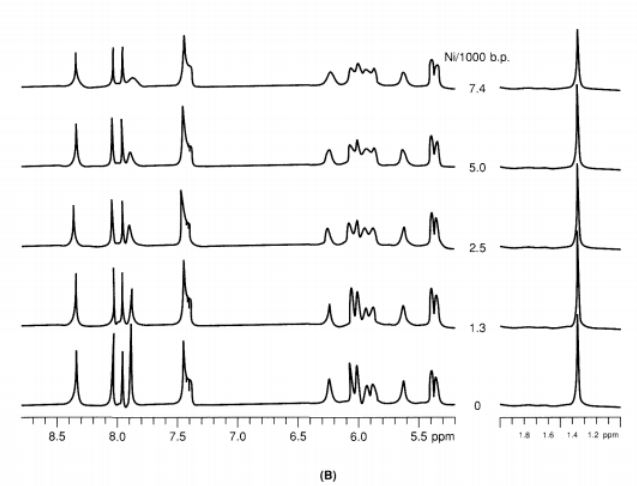


Figure 8.9 - An illustration of two spectroscopic techniques used to probe DNA. (A) The variation in luminescence characteristics of $Ru(phen)_3^{2+}$ with DNA binding. Shown is the emission spectrum of free $Ru(phen)_3^{2+}$ (----), *Λ-Ru(phen)$_3^{2+}$ in the presence of DNA* (•••••), *and Δ-Ru(phen)$_3^{2+}$ in the presence of DNA* (——) *illustrating the spectroscopic perturbation with DNA binding as well as the associated enantioselectivity in binding of the complexes to the helix. As is evident from the greater luminescence of the Δ isomer on binding, it is this Δ-isomer that intercalates preferentially into the right-handed helix. (B) An application of paramagnetic broadening by metal complexes in NMR experiments to obtain structural information on their association with nucleic acids. Shown is the ^{1}H-NMR spectrum of d(GTGCAC)$_2$ with increasing amounts of Λ-Ni(phen)$_3^{2+}$. Note the preferential broadening of the adenine AH2 resonance (7.8 ppm), indicating the association of this enantiomer in the minor groove of the helix.*

There are numerous other classic techniques of inorganic chemistry that have been or could be applied in studying the binding of metal complexes to nucleic acids. Coordination complexes have invariably been used in x-ray diffraction experiments because of the high electron density of the metal center. The tris(phenanthroline) metal complexes have not yet been applied in this context, but, as mentioned already, platinum metallointercalators were examined by fiber diffraction to delineate intercalation requirements. In fact, many nucleic-acid crystal structures have required specific metal ion additions for isomorphous heavy-metal derivatives to solve the structure. Such has certainly been true for the crystal structure of $tRNA^{Phe}$, where heavy-metal ions such as platinum, osmium, and mercury were targeted to specific base positions, and lanthanide ions were used to label phosphate positions around the periphery of the molecule.[53] Other techniques can also be exploited to monitor and characterize binding. A recent novel illustration is one from electrochemistry, which has been applied in monitoring the binding of $Co(phen)_3^{3+}$ to DNA.[54] Surely other techniques, from EXAFS to scanning tunneling microscopy, will be exploited in the future.

Biochemistry also provides very sensitive techniques that have been invaluable in characterizing interactions of metal complexes with nucleic acids. First are simply gel electrophoresis experiments, which permit an assessment of changes in the nucleic-acid conformation, through its changes in gel mobility, as a function of metal binding. A classic illustration is that of the unwinding of superhelical DNA as a function of intercalation. Closed circular DNA has much the same topological constraints on it as does a rope or a telephone cord; the DNA helices can wind up in coils. We define the duplex turning in a double helix as the secondary helical turns, and turns of the helices about one another as the supercoils or tertiary turns. As long as a DNA double helix is closed in a circle (form I), the total winding, that is, the total number of secondary and tertiary turns, is fixed. Molecules with differing extents of winding have different superhelical densities. In a circular molecule with one strand scission, what we call form II (nicked) DNA, the topological constraints are relaxed, and no supercoils are apparent. The same, by analogy, can be said of a telephone cord off the phone receiver, which can turn about itself to relax its many supercoils. Now let us consider a DNA unwinding experiment, monitored by gel electrophoresis. Supercoiled form I DNA can be distinguished from nicked DNA (form II) in an agarose gel because of their differing mobilities; the wound-up supercoiled molecule moves easily through the gelatinous matrix to the positive pole, whereas the nicked species is more floppy and thus is inhibited in its travels down the gel. A closed circular molecule with no net supercoils (form I_0) comigrates with the nicked species. Consider now the supercoiled molecule in the presence of an intercalator. Since the intercalator unwinds the DNA base pairs, the number of secondary helical turns in the DNA is reduced. In a negatively supercoiled, closed circular DNA molecule, the number of supercoils must be increased in a compensatory fashion (the total winding is fixed); hence the total number of negative supercoils is reduced, and the molecule runs with slower mobility through the gel. As the intercalator concentration is increased still further, the mobility of the supercoiled species decreases until no supercoils are left, and the species comigrates with the nicked form II DNA. Increasing the bound intercalator concentration still further leads to the positive supercoiling of the DNA and an increase in mobility. Figure 8.10 illustrates the experiment with tris(phenanthroline)ruthenium(II) isomers.[46] This kind of unwinding experiment is an example of

the sensitivity with which DNA structural changes can be monitored using biochemical methods; only low quantities (< μg) of materials are needed to observe these effects.

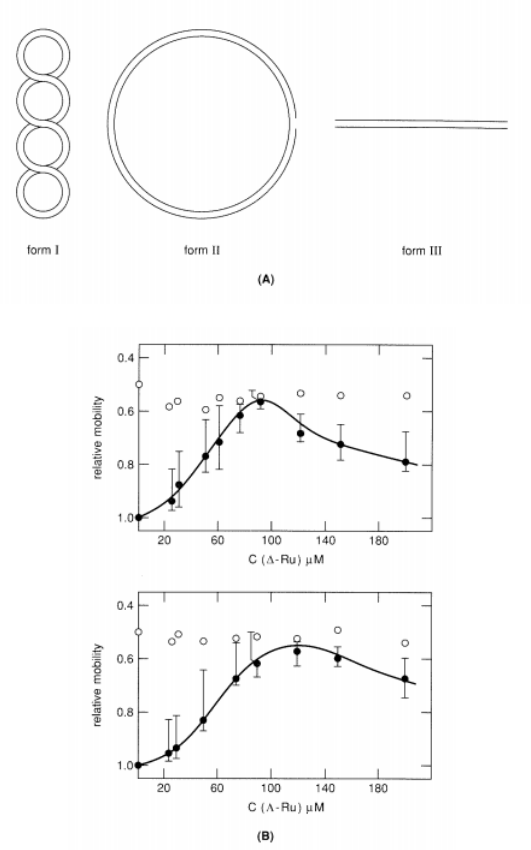


Figure 8.10 - The application of DNA supercoiling to probe metal-complex interactions with DNA. (A) A schematic representation of supercoiled DNA (form I), nicked DNA (form II) that, as a result of the single-strand scission, relaxes to a circular form lacking supercoils, and linear (form III) DNA. (B) Plots of the relative electrophoretic mobilities of form I (●) *and form II* (◯) *DNA as a function of increasing concentration of* Δ*-(top) and* Λ*-(bottom)* $Ru(phen)_3^{2+}$ *in the gel.*[46] *Increasing concentrations of bound intercalator unwind the negatively supercoiled DNA. Given the higher intercalative binding affinity of the* Δ*-isomer, slightly lower concentrations of this isomer are needed to unwind the plasmid to a totally relaxed state (where form I and II comigrate).*

DNA strand scission can also be sensitively monitored, and even more importantly the specific nucleotide position cleaved can be pinpointed by biochemical methods. This methodology has been applied successfully in monitoring both the efficiency of DNA strand scission by metal complexes and the specific sites cleaved, and hence where the complexes are specifically bound on the helical strand.

Relative extents of cleavage of DNA by different metal complexes can be easily assayed in an experiment that is an extension of the unwinding experiment described above. One simply measures the conversion of supercoiled form I DNA to nicked form II species. One strand cleavage on the DNA circle releases the topological constraints on the circular molecule and relaxes the supercoils. Two cleavage events within 12 base pairs on opposite strands will convert the DNA to a linear form (III), which also has a distinguishable gel mobility. Photoactivated cleavage of DNA by tris(phenanthroline) complexes of cobalt(lll) and rhodium(III) was first demonstrated using this assay.[55,56] Given the high sensitivity of this assay, redox-mediated cleavage of DNA by a wide range of metal complexes can be easily demonstrated. However, other techniques are required to analyze whether appreciable and significant cleavage results, and, if so, what products are obtained. Since the assay can monitor, in a short time using little sample, a single nick in a full 4,000-base-pair plasmid, reactions of very low, almost insignificant yield can be detected. The assay provides, however, a simple scheme to assess *relative* extents of cleavage by different metal complexes, as well as a first indication that a cleavage reaction by a given metal complex occurs at all.

More informative are experiments on ^{32}P-labeled DNA fragments using high-resolution polyacrylamide gel electrophoresis, since these experiments allow one to find the exact nucleotide where the complexes break the sugar-phosphate backbone. Consider a cleavage reaction by a given metal complex on a DNA fragment of 100 base pairs in length that has been labeled enzymatically with ^{32}P on one end of one strand. If the metal complex cleaves the DNA at several different sites, then one can arrive at conditions

where full cleavage is not obtained, but instead a population of molecules is generated where single cleavage events per strand are obtained, and cleavage at each of the sites is represented. After denaturation of the fragment, electrophoresis through a high-density polyacrylamide gel, and autoradiography, only fragments that are radioactively end-labeled are detected, and hence the population of sites cleaved is determined. The denatured cleaved fragments move through the gel according to their molecular weight. By measuring their length, using molecular-weight markers, one can find the specific position cleaved relative to the end of the fragment. By this route the specific sites cleaved by a molecule that binds and cleaves DNA, or end-labeled RNA, at unique positions may be identified. In a complementary experiment, using footprinting, where a molecule cleaves DNA nonspecifically at all sites along a fragment, one can find the binding positions of other molecules such as DNA-binding proteins. In this experiment, cleavage with the sequence-neutral cleaving reagent is carried out both in the presence and in the absence of the other binding molecule. In the absence of the protein, cleavage ideally occurs at all sites; hence a ladder of cleaved fragments is apparent on the autoradiograph. If cleavage is carried out in the presence of the protein, however, those sites that are bound by the protein are protected from cleavage by steric considerations, producing a shadow or footprint of the protein-binding site on the gel. Both the site-specific and footprinting experiments are illustrated schematically in Figure 8.11. This very powerful technology was first applied by Dervan and coworkers in demonstrating the application of methidium-propyl-FeEDTA (MPE-Fe) as a chemical footprinting reagent.[22,57] Tris(phenanthroline) metal complexes have been shown to cleave DNA nonspecifically, and their derivatives have been applied either as sensitive photofootprinting reagents, or as site-specific cleaving molecules, as we will see.

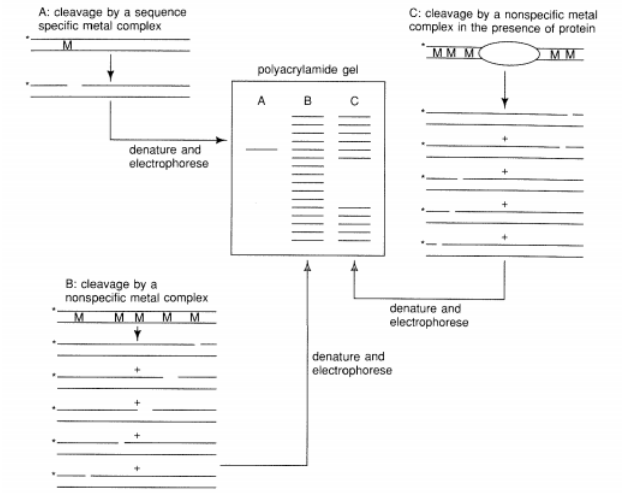


Figure 8.11 - DNA cleavage by metal complexes. Shown schematically is the method used with single-base resolution to discover the sites where metal complexes are bound on double helical DNA. After the metal complex is bound to several sites on a radioactively end-labeled (*) DNA fragment and activated to permit strand cleavage at the binding sites, the nicked DNA is denatured and electrophoresed on a high-resolution polyacrylamide gel, and the gel submitted to autoradiography. From the molecular weights of the end-labeled denatured fragments, the positions of cleavage and therefore binding by the metal complex may be deduced. The results in lane A show the cleavage pattern observed for a metal complex that binds to a specific site. The results in lane B show cleavage observed for a nonspecifically bound metal complex that binds and therefore cleaves at every base site. Lane C illustrates a footprinting experiment. When protein is bound to DNA at a specific site, it protects the DNA from cleavage by the metal complex at its binding site, thus producing a "footprint" in the gel: the absence of end-labeled fragments of those lengths that are protected from cleavage as a result of protein binding.