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Design, Synthesis, and Characterization of Nucleosomes containing Site-specific DNA Damage

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Abstract

How DNA damaged is formed, recognized, and repaired in chromatin is an area of intense study. To better understand the structure activity relationships of damaged chromatin, mono and dinucleosomes containing site-specific damage have been prepared and studied. This review will focus on the design, synthesis, and characterization of model systems of damaged chromatin for structural, physical, and enzymatic studies.

Graphical Abstract

Keywords

Histones; nucleosome; dinucleosome; chromatin; linker; DNA damage; DNA repair; photocaged; DNA photoproducts; AP sites; solid phase DNA synthesis

1. Introduction

Recognition and repair of damaged DNA is critical to the survival of cells and how this occurs when DNA is assembled into nucleosomes in chromatin is of fundamental interest [1–7]. Nucleosomes are the basic building block of chromatin and consist of about 147 bp of DNA wrapped almost two times around a histone octamer containing two each of H2A, H2B, H3 and H4 proteins with a pseudo C2-axis of symmetry (Fig. 1) [8–11]. In euchromatin the nucleosomes are arranged in a loose manner whereas in heterochromatin

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Conflict of interest statement

None

there is further compaction that occurs upon assembly with histone H1 to form a much more dense structure. When DNA wraps around the histone core particle, each nucleotide has a specific orientation relative to the histone surface (Fig. 2), which may make it more or less accessible to a repair enzyme depending on the mechanism of the interaction. The physical environment around a nucleotide also depends on its translational position within the nucleosome which determines which histone tails it interacts with, which can also influence its reactivity and interactions with histones and other proteins. Because the DNA is bent, it does not have the same conformations or dynamics as DNA free in solution, which also causes it to interact differently with physical agents, small molecules, and proteins.

How does nucleosome structure influence DNA damage formation, and how do repair enzymes detect and repair damage that may be sterically inaccessible and in an altered conformation of DNA? It was not until the first high resolution crystal structure of a nucleosome was reported in 1997 by Luger and coworkers that the molecular-level details of the DNA and its interactions with the histones were revealed [12]. It was just shortly thereafter that Smerdon and coworkers reported the preparation of a nucleosome containing a cis-syn cyclobutane thymine dimer in a fixed rotational and translational setting, thereby paving the way to answer such questions [13]. The ability to prepare nucleosomes containing DNA with well-defined translational and rotational settings as well as those without, has led to a better understanding of the effects of nucleosome structure on DNA damage formation, recognition, and repair, as well as the effect of DNA damage on nucleosome structure. This review will focus on the selection, design, construction, and characterization of nucleosomes containing site-specific damage.

2. Sources of nucleosomes and histones

The preparation of nucleosomes and polynucleosomes containing site-specific DNA damage involves assembling a histone octamer with site-specifically damaged DNA. This can be done by either exchanging the DNA with that in a nucleosome isolated from a natural source, or by assembling the modified DNA with histone octamers isolated from nucleosomes or prepared from expression vectors. It is also now possible to prepare histones by semi-synthesis [14] opening the way to studying the effects of specific histone modifications. The organism from which nucleosomes are obtained is not generally as important as the sequence of the DNA, since histone are highly conserved and assemble with DNA in a similar way [15]. Sizable differences have been found, however, between the crystal structures of frog and human nucleosomes [16].

2.1 Natural sources

To insure that a properly assembled and highly homogenous population of nucleosome core particles is formed, a number of groups have made use of nucleosome core particles isolated from chicken erythrocytes. Unlike mammalian erythrocytes, chicken erythrocytes contain a nucleus, and mature cells are transcriptionally dormant [17] resulting in a fairly homogeneous population of unmodified histones. Even so, some fractions of the histones are modified by acetylation and methylation [18, 19]. Highly homogenous populations of unmodified chicken erythrocyte histone core particles can, however, be isolated [20] and have been of a purity that can be used for crystallographic studies [21]. The nucleosomes are

obtained by digestion of the chromatin with micrococcal nuclease (MNase), after which the linker histones are removed. Alternatively, the histones can be extracted from the nucleosomes and reassembled into an octamer. Nucleosome core particles isolated from HeLa cells have also been used for the preparation of nucleosome core particles but these are likely to be much more heterogeneous in histone variants and modifications [22, 23].

2.2 Recombinant sources

Another widely employed approach for preparing nucleosomes has been to use expression vectors to produce unmodified histone proteins which are then assembled into histone octamer core particles, and then complexed with DNA. The advantage of this approach is that homogeneous proteins are used, though special care must be taken to insure their proper assembly into a nucleosome. Another advantage is that recombinant proteins can be site-specifically mutated or modified to investigate the role of individual amino acids and modifications on the structure and interactions of the nucleosome [24]. In addition, histones can be mutated to allow for the site-specific attachment of reporter groups, such as hydroxyl radical footprinting agents [25], or reactive DNA damaging agents [26]. One of the most extensively used sources of recombinant histone proteins is the frog *Xenopus laevis*, and nucleosomes prepared from these histones have been used for many fundamental x-ray crystallographic studies of nucleosome structure [10]. Generally, the *Xenopus* proteins are expressed as full length proteins without affinity tags [27, 28]. Recombinant histones have also been expressed with his-tags which are used for affinity purification of the individual histones, or their complexes, after which the his-tags are removed enzymatically [29]. There are now also many commercial sources of recombinant histones and histone core particles, which greatly facilitates the preparation of site-specifically damaged nucleosomes for study.

3. Nucleosomal DNA sequences

The sequence of the DNA used to prepare a nucleosome containing site-specific DNA damage depends on whether or not a rotationally and translationally fixed DNA sequence is desired. Early studies had identified a number of naturally occurring DNA sequences that bind to nucleosomes with a preferred rotational orientation and translational position such as the 5S ribosomal RNA gene, and alpha satellite DNA. Later studies employed artificial sequences that were either designed or evolved to have a high degree of rotational and translational phasing.

3.1 5S rDNA

A preferred rotational setting for the 5S rDNA sequence was demonstrated by hydroxyl radical footprinting [30, 31]. MNase digestion of 214 bp section of the *Xenopus borealis* somatic 5S ribosomal RNA gene, however, revealed the presence of at least five translational settings, with 40%, 27%, 20%, 7% and 6% occupancies when assembled with chicken erythrocyte histones [31]. While a 146-mer *Lytechinus variagatus* 5S rDNA initially assembles with two translational settings, subsequent annealing by heating results in a single setting [25, 32]. This is not true, however, with a 180-mer sequence which exists with two translational setting after heat annealing [25]. At least two translational positions were also detected in a dinucleosome construct using two tandem 5S rDNA sequences for studies of

(6-4) photoproduct repair [33]. The lack of strong translational positioning suggests that this sequence may not be the best for investigating DNA damage recognition and repair in di- or polynucleosomal arrays. Another potential drawback for detailed structure-activity studies is that there are no crystal structures of 5S rDNA- containing nucleosomes.

3.2 Alpha satellite DNA

A large number of nucleosome crystal structures have been successfully solved for palindromic or pseudo palindromic forms of alpha satellite DNA from centromeres [34], which has also shown strong rotational and translational positioning. In the first reported high resolution crystal structure, a 146-mer palindromic DNA sequence was used, but unfortunately the axis of symmetry of the nucleosome turned out to lie along a central base pair (1AOI.pdb) [12]. As a result the 146-mer DNA could not fit symmetrically into the nucleosome core particle, having 73 bp to one site and 72 bp to the other side of the dyad axis. To solve this asymmetry problem, a pseudo palindromic 147-mer sequence was designed with an asymmetric central base pair which resulted in a higher resolution structure (1kx5.pdb) [35]. The palindromic alpha satellite sequences have been used by Greenberg and coworkers to construct nucleosomes containing single and multiple oxidative damage sites [36, 37]. Lippard and coworkers, however, have used unsymmetrical sequences bearing only half of the alpha satellite DNA for repair studies of platinum adducts [38–40].

3.3 TG motif

When studying how DNA wraps around nucleosomes, Crothers and coworkers discovered that rotationally phased nucleosomal DNA could be obtained with a synthetic sequence composed of alternating (A/T)₃ and (G/C)₃ blocks separated by two nucleotides, (A/T)₃NN(G/C)₃NN, otherwise known as the TG motif [41]. Footprinting studies were consistent with a model in which the DNA was oriented so as to position the minor groove of the (A/T)₃ block and the major groove of the (G/C)₃ block towards the histone surface (Fig. 2B). This system was used by Li and Wrang to demonstrate the effect of rotational and translational position on transcription factor binding to the glucocorticoid response element [42], and subsequently by Smerdon and coworkers for studying the repair of cis-syn cyclobutane pyrimidine dimers [13, 43] and uracil in DNA [44, 45]. There is no crystal structure of a TG motif containing nucleosome, and the structural details are inferred from footprinting data and by comparison with known crystal structures. It is not clear if a 10-mer repeating sequence is ideal, however, since it was found that while the flanking sequences have a helical repeat of about 10 according to hydroxyl radical footprinting, the central 30 bp portion of a nucleosome sequence is calculated to have a repeat of about 10.7 [30, 46].

3.4 Widom 601 sequence

In another approach to artificially position nucleosomes, Widom and coworkers used SELEX (systematic evolution of ligands by exponential enrichment) to identify high affinity binding sequences to chicken erythrocyte histone octamers [47]. SELEX is a technique in which a library of DNA molecules containing known flanking sequences and a random core is incubated with a target ligand after which the bound DNA is separated, and amplified by PCR. The amplified DNA is then resubjected to binding and amplification multiple times, cloned and sequenced. One nucleosome binding sequence obtained by this procedure,

known as the Widom 601 sequence, forms an extremely stable rotationally and translationally phased nucleosome. The structure of the 601 sequence in a complex with *Xenopus laevis* histone octamer has recently been solved by x-ray crystallography (3LZ0.pdb) [48]. Because of its stability, asymmetry, and availability of a crystal structure, the Widom 601 sequence has become the preferred choice for most recent studies of site-specific DNA damage [49–53]. There are differences between the conformation seen in the alpha-satellite and 601 sequence nucleosome structures. Most notably, the 601 sequence forms a 145 bp nucleosome while the alpha satellite DNA forms a 147-mer nucleosome. In addition, anomalous repair kinetics have been observed for 601 nucleosomes, suggesting the presence of multiple conformations in the nucleosome [32]. In spite of its strong affinity and positioning properties *in vitro*, other factors have been found to control nucleosome formation on this sequence in yeast [54].

3.5 Unphased sequences

It is not always desirable to have a nucleosomal sequence with strong translational or orientational preference. Most nucleosomes in human DNA are not strongly positioned [55] and it is of interest to know whether a particular type of DNA damage may induce specific orientational and translational positioning. For example, will the DNA damage prefer to face outwards or inwards, or might it want to reside in the linker rather than in the nucleosome core. Some groups have used sequences that do not form strongly oriented or positioned sequences to answer these questions [56–59], but no one has designed a sequence that binds well to core histones without specific rotational or translational positioning.

4. Site-specific incorporation of DNA damage into nucleosomal DNA

There are a number of strategies for the construction of site-specifically damaged nucleosomal DNA, which are largely based on methodology previously developed for preparing DNA substrates for *in vitro* and *in vivo* DNA repair and replication studies (Fig. 3). One approach relies on the chemical synthesis of a phosphoramidite DNA damage building block that can be used to incorporate DNA damage into a fragment of nucleosomal DNA by standard automated DNA synthesis for subsequent ligation, or even into full length nucleosomal DNA. As an example of the latter approach, uracil has been site-specifically incorporated into 150-mer oligonucleotides by automated DNA synthesis [45]. The potential drawback of total automated synthesis of nucleosomal DNA is that the yield is generally low, and the purity of the product may be questionable because repetitive deblocking steps with acid causes depurination [60], and in addition may degrade the DNA lesion being incorporated into the sequence. Recently, milder deprotection conditions have been developed to allow 150-mers to be synthesized in fmol amounts with minimal depurination [61]. Most often, shorter ODNs containing the site-specific DNA damage are ligated to DNA duplexes prepared either by automated synthesis, as used for the incorporation of a cyclobutane thymidine dimer into nucleosomal DNA [13], or excised from plasmid DNA, as used to incorporate 8-oxoG [49]. The ligation strategy is often necessary when other reporter groups, such as FRET pairs, need to be incorporated into the DNA [62]. A shorter ODN can also be used to prime DNA synthesis on a single strand circular phage and the nucleosomal DNA excised by restriction digestion as has been used by Hanaoka and coworkers for

incorporating the (6-4) TC photoproduct [33]. Such an approach would be very useful for the construction of much longer DNA substrates needed for the preparation of dinucleosomes and polynucleosomes.

4.1 DNA damage building blocks

The most readily available DNA damage building block for DNA repair studies is the phosphoramidate of dU which is used to incorporate uracil site specifically into nucleosomal DNA [44, 45, 52, 63–66]. Uracil results from the spontaneous deamination of C and is the substrate for repair by the base excision repair enzyme, uracil glycosylase. This enzyme converts deoxyuridine into an apyrimidinic/apurinic or AP site, which is also a ubiquitous type of DNA damage that is a substrate for AP endonucleases and lyases. There are also commercially available phosphoramidite building blocks for other major types of oxidative damage, such as thymidine glycol (TG) and 8-oxodG which have been introduced singly or in pairs to study the effects of clustered lesions that can arise from ionizing radiation [67, 68].

DNA photoproducts have been introduced into nucleosomal DNA by way of DNA synthesis with phosphoramidite building blocks (Fig. 4A and 4B). The first nucleosome core particle containing site-specific DNA damage with a fixed rotational and translational position was carried out by Kosmoski and Smerdon [13] utilizing a phosphoramidite building block for a cis-syn thymine dimer by adapting an earlier synthetic method [69]. A commercial building block for the thymine dimer is available from Glen Research and Cambio but is prohibitively expensive. A TC (6-4) photoproduct has also been introduced into nucleosomal DNA by Hanaoka and coworkers [33] using a phosphoramidite building block developed by Iwai and coworkers [70].

4.2 Photocaged DNA damage building blocks

Some types of DNA damage are inherently unstable and can react with both DNA and proteins. The problem with generating such types of DNA damage before assembly into a nucleosome is that they might degrade or react during the assembly process and lead to products other than those which would have formed had the damage been initially produced in the nucleosome. For example, an abasic site enzymatically produced from a uracil residue might undergo spontaneous or nucleosome catalyzed β -elimination to yield a strand break and/or crosslink prior to, or during nucleosome formation. To avoid this problem, Greenberg and coworkers have been pioneering the use of photocaged DNA damage building blocks to prepare site-specifically damaged nucleosomes for kinetic studies (Fig. 5C–5E). The strategy is to incorporate a photolabile precursor to the DNA damage, which is otherwise stable to the conditions of automated synthesis and nucleosome assembly, into the DNA, and then convert it into the damaged DNA by exposure to light of longer wavelength than can directly photodamage the DNA. This methodology was first reported for the preparation of nucleosomes containing site-specific apyrimidinic/apurinic (AP) sites by using a photocaged precursor which was converted to an abasic site with 350 nm light [36]. This strategy has also been employed to introduce the C4'-hydroxy abasic site which is produced by ionizing radiation and radiomimetic agents such as bleomycin [71]. Likewise,

ribonolactones, which are also produced by oxidative damage, can be produced photochemically following assembly and purification of a nucleosomal core particle [50].

4.3 Post-synthetic modification of ODNs

When a phosphoramidite DNA damage building block is not available or easily synthesized, the required site-specifically modified ODN can be prepared by reacting an ODN containing a unique target site with the mutagen and then isolating the desired damaged DNA product by HPLC. This approach has been successfully carried out for the construction of cis-syn [58] and (6-4) photoproducts of TT [23, 58], cisplatin adducts of GG [38] and GTG [59, 72], and AAF adducts of G [57, 58]. This approach, however, does not easily allow for the study of sequence context effects, which are better studied with a building block approach.

5. Assembly and characterization of nucleosomes containing site-specific DNA damage

DNA bearing site-specific damage is then assembled into a nucleosome by either an exchange reaction with nucleosome core particles isolated from natural sources or by assembly with histone core particles prepared from natural sources or expression vectors. In the former procedure a large excess of nucleosome core particles are incubated at high salt concentration (generally 2M NaCl) with radiolabeled damaged DNA to facilitate dissociation and DNA exchange. The reconstituted nucleosomes containing site-specifically damaged DNA are progressively stabilized by reducing the salt concentration by dialysis [41]. High salt is also used for the assembly of nucleosomes from histone core particles with DNA, and in many cases carrier DNA is added. In some cases, the nucleosome is further heated to about 40°C to equilibrate any less stable translational isomers that may have been trapped during the salt dialysis method [25]. After the assembly is completed, the preparation can be used as is, or can be further purified by velocity sucrose gradient centrifugation to separate the nucleosome from the unbound DNA [23]. For studies in which more compacted forms of chromatin are of interest, the linker histone H1 is added. The linker histone, is not well behaved, however, and requires the NAP1 histone chaperone to assemble it properly into a nucleosome [51, 73], although some studies have simply added H1 to nucleosome preparations [33].

5.1 Chemical and enzymatic footprinting

To determine the degree and orientation of rotational phasing of the nucleosomal DNA, DNase I [74] or preferably hydroxyl radical [75] footprinting is used. Hydroxyl radical footprinting of nucleosome associated DNA gives rise to characteristic clusters of cleavage bands which repeat at approximately 10 bp intervals [30, 41]. Hydroxyl radicals principally attack the H5', 5'' and H4' protons that reside in the minor groove of DNA [76]. In a nucleosome the major cleavage bands correspond to sites in the minor groove of DNA where the phosphodiester backbone is furthest away from the histone surface and are centered approximately two bp to the 3'-side of a base pair whose major groove faces the histone surface, as indicated by the arrows in Figure 2B. To more accurately assign the particular rotational position of base pairs, the footprinting reactions are carried out on both strands, resulting in cleavage patterns that are 3'-staggered by about 3–4 base pairs [41, 73].

Addition of linker histone H1 causes the hydroxyl radical repeat pattern to extend into the linker region and to depress cleavage at the dyad [73]. To assign the translational position and dyad of the nucleosome, internally labeled DNA containing restriction sites is first digested with MNase which will cleave DNA protruding from the ends of the nucleosome [77]. Cleavage of the nucleosome protected fragment with the restriction enzymes then allows the edges of the nucleosome to be mapped. Multiple nucleosome positions are revealed by multiple cleavage bands whose intensities reflect the relative population distribution. Alternatively, 5'-end labeled nucleosomal DNA can be digested from the 3'-end with exonuclease III and the matching cleavage bands assigned to individual nucleosome positions [78].

5.2 Physical methods

The thermodynamic stability and dynamics of site-specifically damaged nucleosomes can be determined by equilibrium binding and fluorescence studies. The apparent equilibrium constants for binding native and damaged DNA to a nucleosome can be determined from gel shift experiments [41], from which the K_d of binding can be obtained, as demonstrated for binding to inside and outside facing CPDs [43]. To determine changes in the dynamics of nucleosomal DNA caused by DNA damage, Duan and Smerdon installed fluorescent energy donors and acceptors into nucleosomal DNA containing site-specific a cis-syn thymine dimer or (6-4) photoproduct and then compared the FRET efficiency with that of undamaged DNA [62].

6. Specific examples

6.1 DNA photoproducts

Early studies with heterogeneous DNA showed a preference for cis-syn thymine dimers to adopt outside positions when assembled into chicken erythrocyte nucleosomes due to the bending that they caused [56]. Indeed, hydroxyl radical footprinting of TT cis-syn and (6-4) photoproducts, site-specifically incorporated into DNA containing no positioning sequences, showed that the nucleosome prepared from a HeLa histone octamer core became phased [58]. In contrast, Smerdon and coworkers showed that cis-syn TT dimers incorporated into inside and outside positions near the dyad of a TG motif (see 3.3), chicken erythrocyte nucleosome maintained their rotational positions [43]. They also found that there was only a 0.5 kJ/mol (0.12 kcal/mol) difference in stabilization between the inside and outside facing CPDs that was not sufficient to override the rotational phasing ability of the TG motif. The nucleosome only slowed down nucleotide excision repair 2–3 times and was only 1.5-times slower for the inward facing CPD (see elsewhere in this issue). By using the same TG motif system, Taylor and coworkers were able to show that the deamination of CPDs of methylated TCG sites thought to be responsible for the origin of UV-induced C to T mutations was strongly modulated by rotational position [79, 80]. By incorporating fluorescent probes into the nucleosomal DNA, Duan and Smerdon were also able to show that CPDs and (6-4) products can enhance unwrapping of DNA from histones [62]. In an early study by Hanaoka and collaborators, (6-4) photoproducts were site-specifically incorporated into the nucleosome centers or linker of a dinucleosome for excision repair studies [33]. DNase I footprinting and MNase mapping indicated that the (6-4) photoproduct

did not alter either rotational or translational phasing of the dinucleosomes. They also found that while excision repair was strongly inhibited in the nucleosomes as well as the linker, it was enhanced in the linker by the ATP-utilizing chromatin assembly and remodeling factor (ACF). On the other hand, Sancar and coworkers found that SWI/SNF chromatin remodeling factor could enhance NER of the (6-4) TT photoproduct but not the cis-syn dimer in a nucleosome [58]. Thus, nucleosomes can modulate the formation and repair of DNA photoproducts depending on their rotational and translational position, and the rotational and positioning strength of the nucleosomal DNA sequence.

6.2 Cisplatin adducts

Cisplatin is a chemotherapeutic agent that acts by forming intrastrand DNA crosslinks by reacting with N7 of G's in the major groove. Cis-[Pt(NH₃)₂]²⁺ 1,3-d(GTG) crosslinks were found to induce strong nucleosome phasing when incorporated into an otherwise weakly phased nucleosome, which was constructed from part of the alpha satellite sequence and a HeLa histone core particle [59]. They were found, however, not to fix the translational position. In a more strongly phased nucleosome constructed from a 5S rDNA sequence and HeLa histone octamer, the cis-[Pt(NH₃)₂]²⁺ 1,3-d(GTG) crosslink was found to override the intrinsic phasing and rotate the DNA so that the platinum adduct in the major groove faces the histone core. A crystal structure of the 1,3-crosslink in a nucleosome constructed with *Xenopus* histones and half of the alpha satellite DNA confirmed the orientation of the adduct [40]. A cis-[Pt(NH₃)₂]²⁺ 1,2-d(GG) crosslink was also shown to override intrinsic nucleosome phasing [38]. In addition, nucleosomes were found to greatly inhibit nucleotide excision repair (NER) of both the 1,3 and 1,2 adducts [72], indicating that phasing promoted by cisplatin restrains nucleosome mobility that could be necessary for efficient repair.

6.3 Base excision repair substrates

A number of studies have been carried out on the repair of various damaged bases (e.g.; uracil, thymine glycol, and 8-oxodG), by base specific glycosylases, which is the first step in the base excision repair (BER) pathway (see elsewhere in this issue). Among the various substrates, uracil has been the easiest substrate to study because the phosphoramidite is commercially available and inexpensive. In an early study, it was reported that although nucleosome formation inhibited uracil glycosylase, repair was relatively independent of both translational and rotational position of the uracil in *Lytechinus variegatus* 5S rDNA assembled onto a chicken erythrocyte histone octamer [66]. Subsequent studies by the Smerdon [44, 45, 52, 65], Hayes [63] and Stivers [64] groups in a variety of different nucleosomes have found otherwise. Generally, outward facing uracils were more rapidly repaired than inward facing ones, and uracils furthest away from the dyad were repaired the fastest indicating that steric accessibility coupled with unwrapping motions are important in repair. Similar results and conclusions have also been arrived at with thymine glycol repair by the NTH1 glycosylase [5, 32, 67]. A correlation was also found between groove width and cleavage rate by uracil glycosylase that was made possible by using the 601 sequence complexed with *Xenopus* histones for which a crystal structure exists [52, 64]. Crosslinking of the DNA to the nucleosome with formaldehyde was found to enhance the difference in repair rates between inward and outward facing uracils, suggesting that rotational dynamics also plays an important role in the accessibility of DNA damage [45]. Addition of linker

histone H1 was shown to slow the rate of repair of uracil, presumably by also altering the dynamics of the DNA [63]. Linker histone H1 was also shown to impede the ability of OGG1 (8-oxoguanine DNA glycosylase) to repair 8-oxoG in the linker of a dinucleosome construct [51]. To probe the effect of nucleosomes on formation of double strand breaks that might arise from BER of clustered base damage of the type that would arise from ionizing radiation, Pederson and co-workers prepared a series of nucleosomes containing site-specific closely opposed thymine glycols and 8-oxoG's [53]. They found that double strand break formation depended on a number of factors including the rotational orientation and spacing of the two DNA lesions, as well as the glycosylase and AP endonuclease involved, and that overall, nucleosomes suppress double strand break formation.

6.4 AP sites

Apyrimidinic/apurinic (AP) sites are common lesions that are produced spontaneously by acid catalyzed depurination, by DNA damaging agents, or by DNA glycosylases during BER. Additionally, AP sites are reactive and can be converted to strand breaks and DNA protein crosslinks (DPC's) [81]. By constructing *Xenopus* nucleosomes containing site-specific single and closely opposed photoactivatable AP sites in either alpha satellite or 601 DNA, Greenberg and coworkers demonstrated that nucleosomes can accelerate the formation of both single and double strand breaks up to 60-fold relative to free DNA and in the process produce protein DNA crosslinks [36, 37]. They also found that the kinetics of these reactions depended on the location of the AP sites and that the cleavage and crosslinking reactions involved histone lysine residues. In studies with photoactivated C4'-oxidized AP sites, single strand breaks were accelerated from 100 to 550-fold, but the accompanying DNA protein crosslinks were unstable and ultimately resulted in transfer of the sugar carbons to the lysine, resulting in modified histones [71]. Thus, in addition to controlling the formation and accessibility of damaged DNA through rotational and translational positioning, nucleosomes can also actively catalyze further reactions of DNA damage resulting in position dependent strand breaks and DNA protein crosslinks.

7. Concluding remarks

The ability to construct mono and dinucleosomes with site-specific DNA damage, coupled with structural and physical studies has enabled a better understanding of the factors influencing the reactivity, detection, and repair of DNA damage in chromatin. The systems and methods that have been pioneered by Smerdon and others will be useful in aiding the design and synthesis of substrates for future studies of clustered lesions, DNA protein crosslinks, and other types of damage.

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Abbreviations

AP apyrimidinic/apurinic

BER	base excision repair
CPD	cyclobutane pyrimidine dimer
DNase I	deoxyribonuclease I
dU	deoxyuridine
FRET	Förster resonance energy transfer
MNase	micrococcal nuclease
NER	nucleotide excision repair
NCP	nucleosome core particle
ODN	oligodeoxynucleotide
8-oxodG	8-oxo-2'-deoxyguanosine
SELEX	systematic evolution of ligands by exponential enrichment

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Highlights

- Mono and dinucleosomes containing single and multiple site-specific DNA damage can now be easily prepared
- Using specifically positioned DNA sequences the effects of rotational and translational position on DNA damage dynamics and reactivity can be determined
- Site-specifically damaged nucleosomes have led to a better understanding of the principles governing DNA damage detection and repair in chromatin

Prologue

I have known Michael for over twenty years, first from a Gordon Conference, and then with a visit to his department to present a seminar on DNA photoproduct structure-activity relationships in 1995. There he introduced me to Chul-Hee Kang, a crystallographer, which ultimately led to the first crystal structure of a thymine dimer in duplex DNA. Mick's pioneering work on DNA photoproduct formation and repair in nucleosomes, and especially seminal paper on the synthesis of a nucleosome containing a site-specific thymine dimer, inspired me to investigate the effect of nucleosome structure on the deamination of C-containing pyrimidine dimers. Mick helped us get started with our studies by providing us with detailed procedures, and has helped guide our experimental designs and our thinking about the photobiology of chromatin through his insightful publications, seminars, and personal discussions.

Fig. 1. Structure of a nucleosome core particle

Cartoon representation of the 1kx5.pdb crystal structure of a pseudopallindromic 147-mer alpha satellite DNA bound to *Xenopus laevis* histone core particle. The numbers correspond to positions of the bases relative to the dyad axis as shown in Figure 2B. The relative orientation of the bases is shown in Figure 2A. Also shown are the superhelical locations relative to the dyad axis. The sites at SHL ± 1.5 and ± 4.5 are sites where the helix is stretched and more reactive to DNA damaging agents. The nucleosome contains two each

of histone H2A (violet and purple), H2B (brown and green), H3 (red and pink), and H4 (orange and yellow).

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Fig. 2. Relative orientation of the bases in a nucleosome at the dyad

A. Wireframe representation. The bases at the dyad of the 1kx5.pdb structure at positions shown in (B) for the blue strand. While both positions 0 and 5 are intermediate positions, the major groove of position 0 faces the histone surface, while the major groove of position 5 faces away from the surface which could explain differences in recognition by repair enzymes.

B. Positions of the bases and grooves relative to the dyad axis. The major groove is indicated by M, the minor groove by m, and the sites of major cleavage by DNase I and

hydroxyl radical on each strand by the arrows. In the nucleosome, the DNA is bent, which results in compression of the major and minor grooves facing the histone core.

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Fig. 3. Strategies for the construction of nucleosomes and dinucleosomes containing site-specific DNA damage

DNA containing site-specific can be prepared by automated DNA synthesis with phosphoramidite building blocks (Fig. 4) or by post synthetic modification of ODNs. ODNs containing site-specifically damaged DNA can then be incorporated into nucleosomal DNA through ligation or primer extension and then incorporated into nucleosomes via an exchange reaction or by assembly with histone core particles. The triangle represents a DNA lesion.

Fig. 4. Phosphoramidite building blocks for incorporating DNA damage into DNA

To incorporate DNA damage into DNA by standard automated synthesis, a damaged nucleotide or dinucleotide must be derivatized with a dimethoxytrityl group (DMT) at the 5'-end and a diisopropylamino- β -cyanoethyl phosphoramidite group at the 3'-end. In addition, any reactive functional groups must be protected with an appropriate group, such as the photodeprotectable nitroaromatic groups employed for phosphoramidites C-E.

A. Cis-syn cyclobutane thymine dimer

- B.** (6-4) photoproduct of TC
- C.** AP site
- D.** ribonolactone
- E.** oxidized AP site