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Brief report

Ultra-violet light induced changes in DNA dynamics may enhance TT-dimer recognition

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ABSTRACT

Short-wave ultra-violet light promotes the formation of DNA dimers between adjacent thymine bases, and if unrepaired these dimers may induce skin cancer. Living cells have a very robust repair system capable of repairing hundreds of lesions every day. Although many of the details of the dimer repair mechanism are known, it is still a mystery how the dimers are recognized. Because the dimers are hidden from repair proteins diffusing in the cell nucleus, it has been surmised that dimer recognition is indirect. In this paper, a new recognition signal is suggested by a theory of the dimer-induced large amplitude, prolonged oscillations in the motion of the two strands in double-stranded DNA molecules. These large amplitude oscillations of the two DNA strands, localized around the dimer will unveil the dimer allowing the repair proteins to bind to the dimer site. The temperature dependence of the recognition rate is correlated with the inter-strand fluctuations and must decrease with decreasing temperature according to the findings in this paper. Moreover the probability for finding a large opening is localized to the dimer neighbourhood and these large openings may play an important role in dimer-repair protein biochemistry.

Biological molecules at physiological temperatures are dynamic structures constantly in motion due to thermal fluctuations in the environment. Recently studies have indicated that thermal fluctuations in the picosecond-to-nanosecond range might play an important functional role in catalysing biochemical reactions [1] and in distinguishing mutant forms of proteins from wild types [2]. It was also recently found that the DNA nucleosome core particle fluctuates between an open and a closed state (lasting hundreds of milliseconds) suggesting that the dynamics of chromatin may play an important role in revealing the DNA to the "outside world" [3]. The role of DNA fluctuations, where the two strands of double-stranded DNA oscillate with respect to each other in a sequence-dependent manner, was used to show [4] that DNA transcription promoter sequences in the T4 bacteriophage open more frequently and with larger amplitudes compared to other similar sequences which do not promote transcription. This finding suggests DNA dynamics play a more important functional role than previously thought. Expressing temperature on the absolute Kelvin scale reveals that double-stranded DNA at physiological temperature is close to its denaturation transition. Therefore, the distance between the two strands exhibits large and fast fluctuations. The characteristic time of these oscillations for a "typical" sequence is of the order of picoseconds, proba-

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bly too short to be recognized by macromolecules like repair proteins that interact with DNA. However, evolution appears to have selected special sequences exhibiting "anomalously" large and prolonged openings to promote a necessary function.

Part of the normal functioning of a cell is the repair of DNA damage caused by endogenous and exogenous factors. One such factor is ultra-violet light with wavelength around 250 nm which promotes the formation of a cyclobutane ring between adjacent pyrimidine bases, usually two thymidines, on the same strand [5] (Fig. 1). If these dimers are not repaired before replication they interfere with DNA synthesis and cause a high rate of mutations [6]. Cyclobutane pyrimidine dimer repair can proceed along several pathways in different organisms: repair by photoreactivation, base-excision repair, or nucleotide-excision repair. In human cells, nucleotide-excision repair (NER) is the only known mechanism for dimer removal. The first step in the repair process is the recognition of a dimer by the repair enzymes. DNA base pairs, being on the inside of the double helix, are hidden from repair proteins diffusing in the cell nucleus and therefore direct contact with the dimer is not possible. NMR experiments [7,8] and molecular-based calculations [9–11] have revealed that, in DNA fragments, the dimer causes local deformations of the DNA molecule suggesting a possible mechanism of dimer recognition. Specifically, it has been suggested that the change in local curvature caused by the dimer is sensed electrostatically by an enzyme which binds to the lesion. Recently,

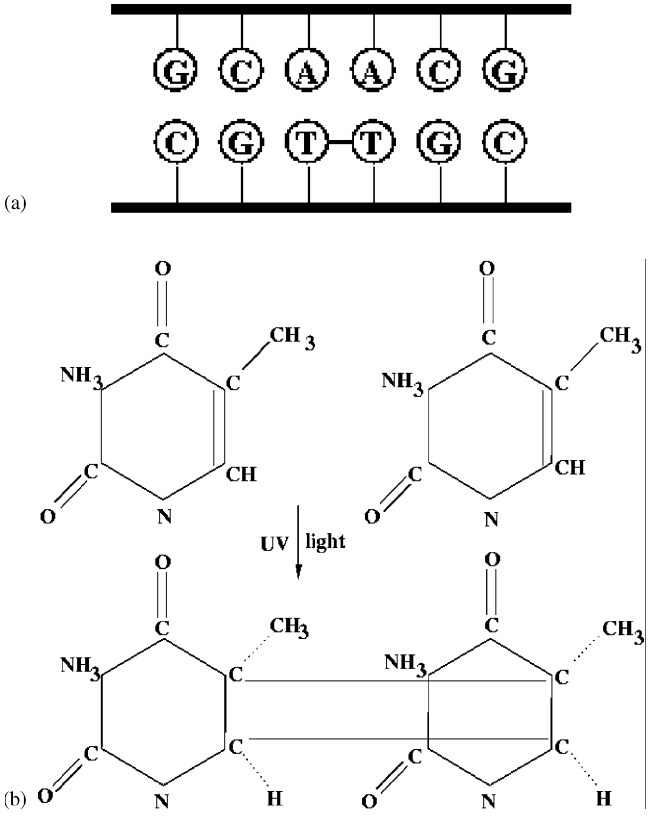

Fig. 1 – (a and b) 250 nm ultra-violet light creates a quasi-stable dimer configuration with a long life-time. The dimer must be repaired fast to ensure that mutations do not propagate after cell division.

based on NMR data, a dynamic dimer recognition mechanism (and more generally for a mechanism for lesion recognition in the NER pathway) was proposed based on the conformational flexibility of DNA in the neighbourhood of the lesion [12]. In the human nucleotide-excision repair pathway, six repair proteins have been identified that participate in the excision, but in vitro none of them can distinguish the UV-damaged from the normal DNA. This has prompted Reardon and Sancar [13] to invoke the principles of thermodynamic cooperativity and kinetic proofreading to explain dimer recognition in humans. Until now it has been assumed that the dimer itself is recognized by the NER complex. However, recent experiments [14,15] suggest that this might not be the case. These experiments suggest that the recognition signal is sent by the intact strand and not by the strand containing the dimer. In this paper, based on Monte Carlo and Langevin dynamics simulations of a specific model, an additional mechanism of dimer recognition is proposed. It is shown that dimer formation leads to large and prolonged openings of the two DNA strands at the dimer location. We propose that these "bubbles" both serve as a lesion recognition signal and expose the dimer to repair proteins. It is interesting to note that the mechanism proposed in Ref. [14] is consistent with our findings because the strand containing the dimer is less flexible than the undamaged strand. The large openings at the dimer site reflect the large oscillations of the intact strand with respect to the lesion containing strand. Whereas bubble formation is strongly temperature-dependent, dimer-induced deformation of local structure is not, possibly providing a means to discriminate between the two proposed dimer recognition mechanisms.

The mechanism of dimer recognition proposed here is similar to the mechanism of binding of a ligand to a structurally inaccessible (in the ground state) cavity mutant of the T4 lysozome, L99A [16]. In this case, dynamic fluctuations allow ligand entry into the otherwise inaccessible cavity. We also note that the DNA repair proteins poly ADP-ribose polymerase and DNA-dependent protein kinase bind to so-called base unpairing regions that, because of a high AT base pair content, have a natural tendency to form bubbles [17].

The dynamic properties of double-stranded DNA are well described by the Peyrard–Bishop–Dauxois (PBD) model [18,19]. This phenomenological model was developed in the late 1980s to describe the nature of the observed denaturing transition in DNA melting experiments. Because of its ability to describe the denaturation transition in double-stranded DNA, the PBD model is suitable for studying the effect of dimers. Dimer formation (Fig. 1) causes adjacent thymine base pairs to form a strong covalent bond while pairing with the complementary adenines is weakened [7]. In the double-stranded octamer d(GCGTTGCG)·d(CGCAACGC) the AT pairing is substantially weakened leading to a drop of the melting temperature by 13 K upon thymine dimer formation. This suggests that the DNA dynamics in the presence of dimers will be modified in the neighbourhood of the dimer in a longer DNA sequence. The study in this paper was made for a 72 base pair DNA fragment in which the d(GCGTTGCG).d(CGCAACGC) octamer is embedded in the middle and the rest of the base pairs are chosen randomly. The thermodynamic properties of the DNA fragment were extracted from Monte Carlo simulations in which the parameters in the model were adjusted to reproduce the