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DYNAMICS OF THE *ESCHERICHIA COLI* NUCLEOTIDE EXCISION REPAIR SYSTEM

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Summary. - *Ultraviolet light induced pyrimidine dimers in DNA are recognized and repaired by a number of unique cellular surveillance systems. At the highest level of complexity* Escherichia coli (E. coli) *has a* uvr *DNA repair system comprising the UvrA, UvrB and UvrC proteins responsible for incision. There are several preincision steps governed by this pathway which includes an ATP-dependent UvrA dimerization reaction required for UvrAB nucleoprotein formation. This complex formation driven by ATP binding, is associated with localized topological unwinding of DNA. This protein complex can catalyze an ATP-dependent 5'→3' directed strand displacement of D-loop DNA or short single strands annealed to a single stranded circular or linear DNA. This putative translocational process is arrested when damaged sites are encountered. The complex is now primed for dual incision catalyzed by UvrC. The remainder of the repair process involves UvrD (helicase II) and DNA polymerase I for a coordinately controlled "excision resynthesis" step accompanied by UvrABC turnover. Furthermore, it is proposed that levels of repair proteins can be regulated by proteolysis. UvrB is converted to truncated UvrB* by a stress induced protease which also acts at similar sites on the* E. coli *Ada protein. Although UvrB* can bind with UvrA to DNA it cannot participate in helicase or incision reactions. It is also a DNA-dependent ATPase.*

Riassunto (Dinamica del sistema del riparo per escisione dei nucleotidi in *Escherichia coli*). - *I dimeri di pirimidina indotti da luce ultravioletta nel DNA sono riconosciuti e riparati da un certo numero di sistemi di "sorveglianza cellulare". Le cellule di* Escherichia coli (E. coli) *presentano un sistema di riparazione del DNA* uvr *che conprende le proteine responsabili del processo di incisione, UvrA, UvrB e UvrC. Numerosi processi preincisione sono regolati da questo meccanismo che comprende una reazione di dimerizzazione dell'UvrA ATP-dipendente che è richiesta per la formazione della nucleoproteina UvrAB. La formazione di questo complesso guidata dal legame con l'ATP è associata con lo srotolamento topologico localizzato del DNA. Questo complesso proteico può catalizzare uno spostamento ATPasi-dipendente, in direzione 5'→3', delle eliche del "D-loop" del DNA o di corti filamenti appaiati a DNA a singolo filamento circolare o lineare. Questo processo si blocca in presenza di siti danneggiati. A questo punto il complesso è attivato per un processo di doppia incisione catalizzata dall'UvrC. Il resto del processo di riparazione coinvolge la UvrD (elicasi II) e la DNA polimerasi I per un processo di "excisione-risintesi" controllato in maniera coordinata e accompagnato da un turnover del complesso UvrABC. Inoltre proponiamo che i livelli delle proteine del riparo possono essere regolati dalla proteolisi. UvrB è convertito nella forma troncata UvrB* da una proteasi indotta dallo stress che agisce anche in siti simili alla proteina Ada di* E. coli. *Sebbene UvrB* possa legarsi con UvrA al DNA non può partecipare alle reazioni dell'elicasi o dell'incisione. UvrB è una ATPasi DNA-dipendente.*

The molecular basis of DNA repair is best understood in bacteria due, in part, to the well defined genetic systems coding and regulating these processes. At this juncture in our knowledge insights derived from bacteria are directly applicable to eukaryotes based on biochemical and gene sequence conservation which have led to the current views of the unity of DNA repair recently promoted by Downes [1]. When DNA repair enzymes catalyzing relatively uncomplicated steps are isolated from mammalian sources their mechanisms of action are similar or identical to their bacterial counterparts. The more complicated multi-enzyme nucleotide excision pathway in *E. coli* acts on DNAs damaged by a spectrum of damaging agents referred to as *bulky adducts*. Although a similar system has yet to be identified in mammalian cells both *E. coli uvr* mutants and DNA repair

deficient human cell lines (xeroderma pigmentosum) show similar survival sensitivities to the same spectrum of bulky adduct damage.

There is an extensive list of DNA damaging agents falling into this category of structural perturbants. It is assumed that these rather large molecular entities cause common conformational distortions since the primary chemical changes are markedly different from each other. The prototype of the bulky adduct is the ultraviolet light-induced cyclobutane dimer between neighboring pyrimidines. Because of the stability of pyrimidine dimers to acid hydrolysis, alkali and nuclease digestion, their temporal fate can be studied during post-irradiation processes both *in vivo* and in crude extracts. These pyrimidine analogs are biologically relevant as pre-mutagenic and pre-carcinogenic lesions [2].

Bulky adducts, in addition to UV-induced cyclobutane dimers and (6-4) photoadducts of pyrimidines include many of the polycyclic aromatic hydrocarbons such as the benzo(a)pyrenes, dialkylbenzanthracenes, aflatoxins, acetyl-aminofluorenes, nitroquinoline oxides, *cis-* and *trans*-platinum, cross-linking agents including mitomycin C, photoactivated psorelen adducts and many of the alkylating agents possessing ethyl or larger alkylgroups.

Nucleotide excision: multiprotein Uvr incision system

Nucleotide excision consists of the four generic biochemical steps, incision, excision, resynthesis and ligation. The incision step is intrinsically the most interesting biochemical step because it is the rate controlling and recognition process in repair. A current working model (Fig. 1) suggests that incision is preceded by rate limiting recognition stages (Fig. 2). Because three separate proteins are required for incision it has been possible to study each partial reaction as a separate molecular event. These four steps are outlined as follows:

step a - *UvrA dimerization*

$$ATP + UvrA \Leftrightarrow 2(UvrA\text{-}ATP)$$

In the first step, ATP causes equilibrium shifts in the molecular weight of UvrA to that of a dimer as well as changing its conformation. This is achieved through nucleotide binding rather than its hydrolysis since ATP-γ-S can stabilize such kinetically transient species.

step b - *Nucleoprotein formation*

$$2(UvrA\text{-}ATP) + DNA \Leftrightarrow [2(UvrA\text{-}ATP)\text{-}DNA]$$
$$[2(UvrA\text{-}ATP)\text{-}DNA] + UvrB \Leftrightarrow [2(UvrA\text{-}ATP)\text{-}UvrB\text{-}DNA]$$

The interaction of the UvrA protein with both undamaged and UV-damaged DNA has been studied both thermodynamically and kinetically. The equilibrium ability

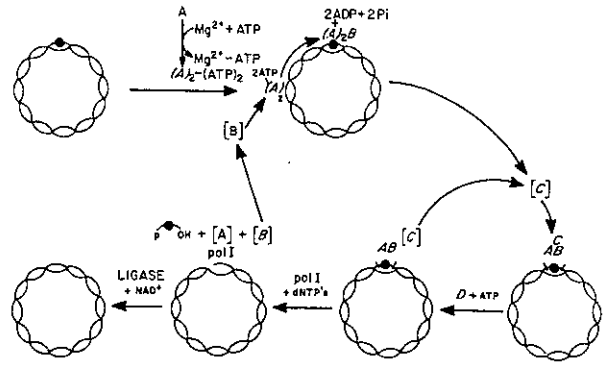

Fig. 1. - The repairosome model of excision repair in *E. coli* which portrays the role of six proteins in incision, excision coupled to resynthesis and ligation. The coupled recycling of UvrC specifically requires UvrD; whereas DNA polymerase I causes the release of UvrB which in turn destabilizes UvrA to complete the repair cycle.

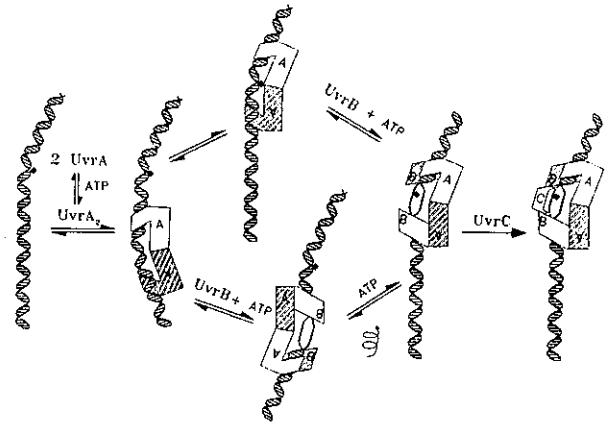

Fig. 2. - The preincision model is visualized as a series of partial reactions emphasizing the mutual allostery associated with the initial dimerization of UvrA, when affected by nucleotide binding. UvrA, as the reactive dimer species, initiates topological unwinding of DNA under the influence of ATP. With the participation of UvrB, unwinding is augmented leading to the UvrAB catalyzed translocation which is driven by the hydrolysis of either ATP or dATP. The repair apparatus stops at the damaged site in preparation for UvrC catalyzed incision.

of the UvrA protein to discriminate between such sites is approximately 10^4 which is surprisingly low given the ratio of undamaged nucleotides to those that are modified. One of the consequences of excess *in vitro* levels of Uvr proteins is dual incision of undamaged DNA; when extrapolated biologically this would be a predicament requiring regulation of enzyme activities.

step c - *Topological unwinding*

$$[2(UvrA\text{-}ATP)\text{-}UvrB\text{-}DNA] + ATP \Leftrightarrow [2(UvrA\text{-}ATP)\ UvrB\text{-}tu^*DNA]$$

* (tu = topologically unwound)

The initial allosteric response (see step a) reflects the influence of nucleotide binding on the conformation and dimerization of UvrA. The effect of Uvr protein binding to DNA is, likewise, accompanied by localized changes in the structure of DNA. The linking number, ΔL which is a measure of supercoiled DNA unwinding, is significantly affected by the binding of UvrA and UvrB to damaged sites by opening up the DNA significantly [3]. This reaction is driven by the binding energy of the nucleotide in which the unwound species represents a kinetically transient state stabilized by either UV damage or by non hydrolyzable ATP analogs.

step d - *Translocation*

$$[2(\text{UvrA-ATP})\text{-UvrB-tuDNA}] + \text{ATP} \Leftrightarrow (\text{UvrAB-tuDNA}^{+}) + \text{ADP} + P_i$$

$^{+}$(damaged site primed for incision by UvrC)

The allosteric effect of the binding of the UvrAB proteins to DNA results in a localized distortion, the dimensions of which are similar to the distance between sites of incision catalyzed by the UvrABC incision system. The influence of single stranded DNA accompanied by the interaction of UvrA and UvrB leads to a significant increase in ATPase activity which appears to be attributable to the cryptic ATPase activity associated with UvrB. This switch in catalytic activity is accompanied by a 5'→3' directed strand displacement process perhaps be having as a translocase or helicase [4]. Given the kinetic preference for damaged sites by UvrA, the persistent or processive movement associated with translocation is perceived as a consequence of the localized topological unwinding of undamaged sites. Since the UvrAB complex binds more tightly to the single stranded regions of undamaged DNA ($\sim 10^8$ vs $\sim 10^6$ for native DNA) there is potential for processive translocation of such a region in which the structure of this region may be preserved and further stabilized when a damaged site is encountered. ATP or dATP hydrolysis probably serves as the essential source of energy for this process.

step e - *Dual endonucleolytic events*

$$(\text{UvrA}_2\text{B-tuDNA}^{+}) + \text{UvrC} \Leftrightarrow \text{UvrA}_2\text{BC-tuDNA}^{+} \rightarrow \text{incised damaged DNA}$$

Unlike the DnaB helicase, the UvrAB strand displacement activity is sensitive to the presence of photochemical damage. A factor in overcoming the indiscriminate specificity is probably a kinetic preference for UvrA off-rates from damaged sites which are minimally three orders of magnitude slower than for undamaged sites. Since ATP hydrolysis is not diminished by the presence of damage, although strand displacement appears to be blocked, it is possible that translocational activity may express its damage sensitivity in two ways. Either the system pauses so it can now be primed for dual incision by UvrC or the complex is able to turnover or "idle" at such sites when UvrC is limiting.

The magnitude of the dual incision event is 7 nucleotides 5'- and 3-4 nucleotides 3'- to the same damaged site [5, 6] which is, significantly, invariant regardless of the nature of the damage [7-10]. These observations imply that the Uvr protein's conformation is able to impose itself on the structure of substrate DNA. Although ATP is required for preincision, evidence suggests that there is no such requirement for the incision reaction.

step f - *UvrD:DNA polymerase I mediated excision*

$$\text{Incised damaged DNA} + \text{UvrD} + \text{polI} + \text{dNTPs} \rightarrow 2\ \text{UvrA} + \text{UvrB} + \text{UvrC} + \text{resynthesized, nicked DNA}$$

The Uvr proteins do not turnover following the dual incision reaction, but remain bound to the incised DNA (Fig. 1). Turnover requires the UvrD protein and DNA polymerase I for a coordinated excision-resynthesis series of reactions in the presence of substrate deoxynucleoside triphosphates: a series of reactions accompanied by UvrABC turnover [11-13]. Because of the physical and functional interaction between these proteins it is suggested that a "repairosome" provides protection to repair intermediates as well as stimulating a coordinated incision excision reaction.

step g - *Ligation reaction: restoration of the integrity to repaired DNA*

Ligation which restores full integrity to the repaired DNA strands is also dependent on prior reactions of UvrD and DNA polymerase [11].

Regulation of DNA repair by proteolysis

Because of the marginal discrimination between damaged and native sites in DNA excess concentrations of Uvr proteins can lead to non specific dual incision of undamaged DNAs. The biological consequence of such indiscriminate activity is, of course, the vulnerability of chromosomal DNA to non specific degradation.

UvrB is converted to UvrB* by a protease (Fig. 3) generated under the stress conditions associated with the amplification of the *uvrB* by temperature induced derepression of the λP_L promoter by the temperature sensitive $\lambda C_I 857$ repressor. UvrB* is generated as a consequence of hydrolysis at a site homologous with protease sensitive sites on the *E. coli ada* induced O^6-methylguanine transferase [14]. This protein, a product of the *ada* gene is required stoichiometrically to remove alkyl groups from alkylation-damaged DNA. It forms a suicide dialkylated protein product which acts as a positive

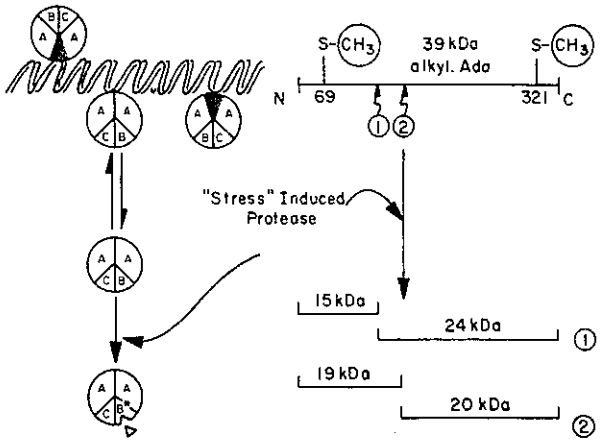

Fig. 3. - The role of proteolysis in DNA repair. UvrB is specifically proteolyzed to UvrB* at sites homologous to the two *ada* protease sites. UvrB* is inactive in nucleotide excision nor can the proteolyzed Ada peptides act as positive transducing signals in *ada* transcription.

transcription inducer to its own synthesis. As a component of this indirect turnover, the alkylated Ada protein is proteolyzed at two unique sites generating four peptides. These same peptides are generated when the Ada protein is exposed to the UvrB protease.

The UvrB protease is stress-induced, under control of the heat shock regulon controlled by the *htpr*+ locus [15]. As a consequence of proteolytic activity UvrB* is generated from UvrB which as a 70 kDa truncated protein can bind with UvrA to DNA but is unable to support the UvrAB helicase or UvrABC incision reactions. Another distinctive feature of this degradative process is that the UvrB protein possesses a cryptic ATPase which becomes apparent on conversion to the UvrB* species. It is this same cryptic ATPase which reveals itself when UvrB interacts with UvrA in the presence of ssDNA. The driving force for the UvrAB induced helicase is the cryptic ATPase associated with UvrB.

Furthermore, it is proposed from these observations that proteolysis induced under conditions of stress serves to help turnover repair proteins in order to minimize those enzymatic excesses when deemed necessary.

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