NEWS AND VIEWS

DNA REPAIR -

Incisions for excision

Jan H. J. Hoeijmakers and Dirk Bootsma

EXCISION implies two incisions. Plumbers and surgeons know this principle, and long ago it was put into practice by the evolutionarily ancient DNA-repair machinery. Dissection of the first incision made by the eukaryotic nucleotideexcision repair pathway has now been described by O'Donovan et al.1, and dissection of the second by Bardwell et al.².

When put together the two processes enable the replacement of a damaged piece of DNA by a new one.

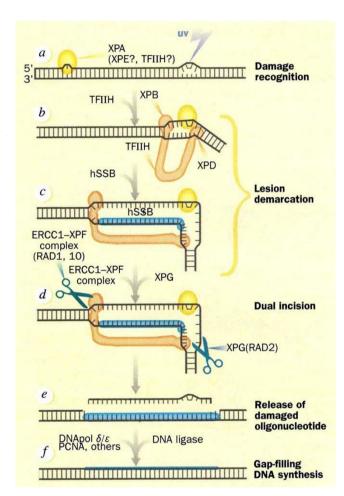
Genetic information is continuously subject to the deleterious effects of environmental agents. The ultraviolet component of sunlight, for instance, promotes dimerization of flanking pyrimidines, which obstructs transcription and upon replication can lead to permanent mutations. The consequences are obvious. Cells may die or (perhaps even worse) suffer from malfunctioning, with carcinogenesis or inborn errors as a final outcome. These consequences are apparent from a class of cancer-prone genetic instability syndromes which stem from a defect in one of the repair systems that nature has acquired to cope with DNA injury.

An example is xeroderma pigmentosum³, which manifests itself as acute sun sensitivity and other skin abnormalities, notably a strong predisposition to skin cancer. Normally sun-induced DNA lesions are removed bv nucleotide-excision repair, a broad-spectrum DNA-repair system. Its biochemical complexity, which was already apparent from the large number of genetic complementation groups identified within

xeroderma pigmentosum, gained an extra dimension with the discovery of two related disorders, Cockayne's syndrome trichothiodystrophy and (otherwise known as brittle hair syndrome). Both of these neurodevelopmental diseases again feature genetic heterogeneity. Thus there are seven xeroderma pigmentosum groups (XP-A to XP-G), two Cockayne's syndrome groups (CS-A and CS-B), three combined XP/CS groups (coinciding with XP-B, XP-D and XP-G) and three trichothiodystrophy groups (TTD-A, XP-B and XP-D), making a total of at least ten

distinct genes involved in nucleotideexcision repair (see our News and Views article of May last year⁴).

Using synthetic 'splayed-leg' and 'bubble' DNA substrates, O'Donovan and colleagues¹ demonstrate that the purified XPG product specifically nicks a duplex at the border of a single-stranded DNA region. Only the strand with the 5' single-



stranded end is touched by this endonuclease. It is likely that the yeast equivalent RAD2 does the same⁵. The yeast RAD1/ RAD10 duo (and probably its human counterpart, the ERCC1-XPF complex) does essentially the opposite: it nicks the strand of which the 3' end is single². Such a structure is present at the other end of a bubble. These results complete the original observations by Huang et al.⁶ that the machinery of nucleotide-excision repair makes a dual incision, one five bases 3' and one 21-23 bases 5' of the lesion.

How do these findings fit into the

nucleotide-excision repair reaction? A tentative scheme is depicted in the figure. The proteins XPA and XPE are likely to be involved in damage recognition, possibly with the assistance of transcription factor TFIIH. The latter was originally known as a multisubunit basal transcription factor. But last year it was shown⁷ that TFIIH also functions in the context of nucleotide-excision repair, and probably does so in its entirety⁸. Lesion recognition presumably induces a conformational change in the DNA helix. Both DNA

'scissors' require single-stranded DNA. so local unwinding around the lesion before incision would seem to be necessary. The TFIIH complex is an excellent candidate for executing this step, because it displays a bidirectional helicase activity conferred by the XPB and XPD subunits⁹. In humans, the melted region provides a niche for SSB, the complex that binds single-stranded DNA and prevents reannealing. After the incisions by XPG at the 3' end and the RAD1/10 complex at the 5' side, the damagecontaining oligonucleotide has to be released. That again may be a task for TFIIH: in basal transcription the complex has been associated with promoter clearance¹⁰, a mechanistically related activity. Finally, the gap of 27-29 nucleotides is filled in by DNA synthesis and ligation.

This model is an oversimplification. For instance, it does not incorporate the CSA and CSB components, which speed up the repair of the transcribed strand of active genes. Also, no account is taken of the XPC-HHR23B complex and the yeast RAD7 and RAD16 proteins, which are involved in control of the (slower) repair of the remainder of the genome.

The findings reported by O'Donovan et al.1 and Bardwell *et al.*² can be placed in a wider clinical perspective. XP-

G is a very rare, heterogeneous form of the disease. Some patients show only features of xeroderma pigmentosum, others additional hallmarks of Cockayne's syndrome. This is a hint that XPG is involved in more than nucleotide-excision repair, and that (depending on the mutation) other processes may become affected. The factors in the transcription/ repair complex TFIIH may present a similar case. Mutations in the XPB and XPD subunits can give rise to symptoms of Cockayne's syndrome and trichothiodystrophy. These features may be due to a

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subtle defect in basal transcription^{4,8}. The XPG symptoms suggest a link with the transcription as well¹, although a firm association with TFIIH could not be shown⁸.

The other scissor, RAD1/10, is also engaged elsewhere. It is required in a mitotic recombination pathway¹¹, where DNA strands need to be cut as well. ERCC1, the human homologue of RAD10, is one of the few proteins involved in nucleotide-excision repair for which no patients have yet been identified. Obviously, most mutations in this gene may be very severe or give rise to an unexpected clinical picture; indeed, ERCC1-deficient mice show a constellation of abnormalities very different from characteristic of xeroderma those pigmentosum¹². One might predict that a category of patients will be found that are deficient in nucleotide-excision repair and also have symptoms of a recombination defect. If these striking multiple engage-

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ments reflect a general evolutionary strategy of function sharing, then intimate connections between nucleotide-excision repair and cell-cycle control or chromatin dynamics are bound to show up as well. \Box

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Baby bunting in paternity probe

Kate Lessells

SHOULD a male work as hard to raise his family if there is a chance that he has been cuckolded? The common-sense answer is 'no', but examples of decreasing paternal care with an increasing chance of cuckoldry have proved elusive. The finding by Dixon et al. (page 698 of this issue¹), that male reed buntings feed their brood

less when a higher proportion of the chicks are genetically fathered by another male, is therefore one of the first clear demonstrations of a relationship between paternal care and 'paternity' (the the chicks proportion of fathered).

Although male reed buntings defend territories containing the nests of one or more pair-bonded females, they are remarkably unsuccessful in defending their paternity: Dixon et al. used singlelocus minisatellite fingerprints to show that 55% of the chicks that they looked at were not the

offspring of the putative father. Moreover, paternity varies enormously from brood to brood. Some variation is bound to occur: even a true coin is unlikely to produce exactly 50% heads when tossed a few times. But paternity is far more variable than the binomial distribution that would be expected if all reed bunting chicks had the same probability of being illegitimate. In other words, this probability varies from brood to brood.

Whatever the cause of this variation, it provides an ideal opportunity to investi-

Reed bunting chick feed; the male adult has a black cap. In contrast, female feeding rate is unrelated to paternity. This confirms that it is the male's lack of genetic relationship with

illegitimate chicks that is the important factor, rather than, for instance, illegitimate chicks requiring less food. The paired comparison between first and second broods is also important because it controls for the possibility that particular males are good both at protecting their paternity and providing paternal care, which has been a problem with previous non-experimental studies². Although the pairwise analysis does not exclude temporary variation in the quality of a male between broods, it does eliminate many confounding variables, such as male age.

One obvious question is what the difference is between reed buntings and other species, for which observational and experimental studies have largely failed to show a relationship between paternal care and paternity³. Mathematical models reveal three situations in which paternal care is not expected to vary with paternity.

First, if the relationship between the total fitness of the brood and paternal care is sigmoidal, there is a threshold level of paternity below which the optimal level of paternal care drops abruptly from some to none⁴. But it is only below this threshold that paternal care should be an unvarying nothing; above the threshold paternal care should increase with paternity.

Second, optimal paternal care will not vary much if the relationship between fitness of the brood and paternal care plateaus abruptly⁵: a slight decrease in paternal care will then result in a precipitous drop in brood fitness, while an increase in paternal care will bring no further benefit. However, the exact shape of this relationship is not known for any species, so this offers no explanatory help with the reed buntings.

Lastly, optimal paternal care is not expected to vary with paternity if individual males have consistently low or high paternity^{5,6}. A reduction in paternal care by heavily cuckolded males frees time and effort for investment in other reproductive attempts, through re-pairing, making extra-pair copulations or simply surviving

to the next breeding season. But if these other attempts are equally cuckolded, paternity will cancel out in an assessment of their value relative to the current brood. Only if the male can expect higher paternity in the future is it worth

him reducing care in the current brood. The reed buntings do indeed exhibit high variation in paternity between broods.

Of course, a male can only modify his paternal care in relation to paternity if he has some way of estimating it. How he does this affects how much variation is needed to explain the relationship

between paternal care and paternity: if he detects the legitimacy of each chick directly, perhaps through some genetic marker, he can respond to even 'coin-tossing'

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gate whether males adjust their brood care to their paternity. Dixon et al. looked at the broods of 13 pairs who bred twice in one season. When the change in male feeding rate is plotted against the change in paternity between first and second broods, a clear relationship emerges: males feed heavily cuckolded broods less.

IMAGE UNAVAILABLE FOR COPYRIGHT REASONS

Dixon, A., Ross, D., O'Malley, S. L. C. & Burke, T. Nature 1. 371, 698-700 (1994). Møller A Anim Behav 36 996-1005 (1988)