Smr: a bacterial and eukaryotic homologue of the C-terminal region of the MutS2 family

Several systems carry out DNA-damage repair in prokaryotes and eukaryotes. One of these, the mismatch-repair (MMR) pathway, recognizes and resolves incorrectly paired nucleotides. It involves a large set of proteins and corrects mainly errors made by the replication machinery, but also mismatched pairings that are the result of recombination events. MutS is central to this pathway: it recognizes and resolves DNA intermediate were to enhance the efficiency of integration/excision in vitro, this finding would support the hybrid hypothesis and implicate tRNA in DNA recombination. Such a role would not be entirely surprising, given that tRNA and tRNA-like molecules are widespread in evolution and, as such, could be used and reused in many different ways in the basal workings of the cellular machinery.

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References


PROTEIN SEQUENCE MOTIFS

to be elucidated. As for other pathogenic islands that are not linked to tRNA genes, it will be interesting to determine whether their flanking sequences encode RNAs that have accessible 3’-ends to participate in the mechanism proposed in the hybrid hypothesis.

The hybrid hypothesis is amenable to experimental analysis. The major prediction is that the presence of the 3’-end of the tRNA encoded by the tRNA gene associated with a pathogenicity island improves the efficiency of integration/excision of the island. Using in vitro studies of bacterial phage integration/excision indeed exhibit poor efficiencies compared with those performed in vivo. In addition to RNA that is complementary to the tRNA encoded by the tRNA gene, it will be interesting to determine the taxonomic distribution: members of the family. The C-terminal region present in members of the family and thereby confirmed the hypothesis.

We searched for new homologues, using the Synechocystis sp. MutS2 sequence as a query for the Blast program (http://www.ncbi.nlm.nih.gov/blast/) and WIT databases. We identified eight new homologous sequences (E = 10^-12 to 10^-20; see Fig. 1), which clearly cluster with the second Synechocystis gene in a phylogenetic analysis (data not shown). This result supports the existence of the mutS2 family. The mutS2 family shows a broad taxonomic distribution; members of the family are present in the Proteobacteria (Helicobacter pylori and Campylobacter jejuni), Gram-positive bacteria (Bacillus subtilis, Clostridium acidiurici/cam and Streptococcus pyogenes), Sphaerotrichia (Borelia burgdorferi), Cyanobacteria (Synechocystis sp.), Cytophagales (Pseudomonas gingivalis) and Aquificales (Aquifex aeolicus). Note that, in all cases, a copy of a member of the mutS2 family is also present, except in the proteobacterial species (H. pylori and C. jejuni). Given that the H. pylori genome has been completely sequenced, the absence of a mutS2 gene in this species is certain and suggests that the product of the unique mutS2 gene functions in mismatched repair. Note that, by contrast, several bacterial species possess only the mutS1 gene.

Members of the MutS2 family have distinct structural features. They are slightly larger than the MutS1 proteins: MutS2 proteins possess 740–820 residues; MutS1 proteins possess 810–910 residues. Members of each family display a high degree of sequence conservation. However, if members of both families are compared, only the central region, which contains the nucleotide-binding domain, can be aligned. Interestingly, members of the MutS2 family lack the –300-residue N-terminal region present in members of the MutS1 family; by contrast, members of the MutS1 family lack a stretch of ~250 residues that corresponds to the C-terminal domain present in members of the MutS2 family. The C-terminal region of MutS2 proteins (the last 90 residues) shows a high degree of conservation, even between distantly related species. Such conservation is not shared by MutS2 proteins which have an important function that is not shared by MutS1.
proteins. To investigate whether species that possess only genes of the mutS2 family lack this conserved sequence completely, we searched the non-redundant NCBI protein database, using the 90 residue C-terminus of the E. coli MutS2 sequence as a query for an iterated PSI-Blast search (http://www3.ncbi.nlm.nih.gov/blast/blast.cgi?cmd=blastp&submit=Search) with default parameters.

Interestingly, among the sequences that produced significant alignments after the first iteration, we identified a small (~200 residue) protein in E. coli and T. pallidum, which contains this conserved region (E-values were $10^{-2}$ and $10^{-3}$, respectively). When these two sequences are included in the PSI-Blast query for the second iteration, together with the MutS2 sequences, a second E. coli homologue is retrieved with a significant score ($E = 10^{-5}$). A likely homologue is also detected in H. influenzae, at least 80% of the sequences are shown against a red background. A green background indicates a conserved chemical character (either hydrophobic (A, C, I, L, M, V, F, W or Y) or polar (D, E, H, K, R, S, T, N or Q)) in at least 60% of the sequences. A blue background indicates that small residues (A, G, S, T, D, N, V, P or H) are conserved in at least 60% of the sequences. Statistically significant motifs in the alignment are indicated (I–IV). aNCBI accession number; A. actinomycetem., A. actinomycetemcomitans; bWIT accession number.

Figure 1

Multiple alignment of the sequence shared by the MutS2 and Smr families. Numbers on the left and right correspond to the distances from the protein N- and C-termini, respectively. Numbers in brackets indicate the length of insertions present in several sequences. Positions that are identical in >90% of the sequences are shown against a red background. A green background indicates a conserved chemical character (either hydrophobic (A, C, I, L, M, V, F, W or Y) or polar (D, E, H, K, R, S, T, N or Q)) in at least 60% of the sequences. A blue background indicates that small residues (A, G, S, T, D, N, V, P or H) are conserved in at least 60% of the sequences. Statistically significant motifs in the alignment are indicated (I–IV). aNCBI accession number; A. actinomycetem., A. actinomycetemcomitans; bWIT accession number.

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How color visual pigments are tuned

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The absorption maximum of the retinal chromophore in color visual pigments is tuned by interactions with the protein (opsin) to which it is bound. Recent advances in the expression of rhodopsin-like transmembrane receptors and in spectroscopic techniques have allowed us to measure resonance Raman vibrational spectra of the retinal chromophore in recombinant visual pigments to examine the molecular basis of this spectral tuning. The dominant physical mechanism responsible for the opsin shift in color vision is the interaction of dipolar amino acid residues with the ground- and excited-state charge distributions of the chromophore.

THE RETINA CONTAINS two types of cells specialized for light detection: rod cells for dim-light vision and three classes of cone cells for color vision. The cones contain pigments absorbing in the blue (~425 nm), green (~530 nm) and red (~600 nm) regions of the spectrum[1,2], whose differential responses enable color vision. These visual pigments consist of an apoprotein (opsin) and an 11-cis-retinal chromophore that is bound to opsin by a protonated Schiff base (PSB) linkage[3] to a specific lysine residue (Lys296). Absorption of light triggers the femtosecond isomerization of the chromophore[4], producing the active signaling state that leads to hyperpolarization of the photoreceptor cell membrane and generation of a visual nerve impulse[5].

Since Isaac Newton's famous treatise on color vision[6], a fundamental aim in vision research has been the elucidation of the factors that determine the absorption maximum of the opsin-bound chromophore. Although the PSB of 11-cis-retinal absorbs at 440 nm in organic solvents, vertebrate color pigments provide environmental perturbations that tune the absorption maximum of the retinal chromophore over a very wide range – from 360 to 635 nm. This shift in the wavelength of maximum absorbance is called the opsin shift. The ~60 nm shift typically observed upon protonation of retinal Schiff bases[7] is not sufficient to explain the red-shifted absorption maxima of green and red visual pigments. Chromophore–protein interactions that might cause this opsin shift include: (1) a weakening of the interaction between the positive charge of the retinal PSB and its negative counterion[8] or hydrogen-bonding partner[9,10]; (2) placement of full or partial charges[11,12] or polarizable groups[13,14] close to the polyene chain; and (3) planarization of the polyene chain caused by the protein environment[15].

Resonance Raman vibrational spectroscopy

The cloning and expression of visual pigments and mutant pigments[16–18] identified Glu113 as the primary cause of the opsin shift mechanism. By combining site-directed mutagenesis with resonance Raman vibrational spectroscopy, we can now compare the vibrational structures of these new proteins with integrated biochemical and genetic approaches.

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