been considered are hydrophilicity, chain termination, mobility, accessibilty amphipathicity and evolutionary variability. Since all these properties of antigens are interconnected, it is probably futile to search for a single primary correlate to 'explain' antigenicity.

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Genetically engineered analogues of human α_1-antitrypsin

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The primary function of α_1-antitrypsin is the inhibition of neutrophil elastase, a protease capable of destroying connective tissue. A α_1-antitrypsin deficiency results in lung emphysema. The α_1-antitrypsin from lungs of individuals who smoke is partially inactivated due to oxidation of the Met residue at the active site of the protein, explaining the high incidence of emphysema associated with cigarette smoking. An ($Met^{358} \rightarrow$ Val) α_1-antitrypsin analogue which remains fully active as an elastase inhibitor but which is also resistant to oxidative inactivation has been constructed and characterized. The properties of other analogues with modified residues in the active site will also be described; the inhibition of thrombin and cathepsin G has been especially studied.

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Classical genetics and site directed mutagenesis in the study of the specific interaction with DNA of CAP, the cyclic AMP receptor protein in *E. coli* K12

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The catabolite gene activator protein (CAP), complexed with its allosteric effector, cyclic AMP (cAMP), plays a key role in gene expression in *E. coli*. It binds to specific DNA sites at or near promoters, where it stimulates the initiation of RNA synthesis. Three mutations that alter the DNA sequence specificity of the catabolite gene activator protein CAP have been isolated, as suppressors of mutations at positions 7 and 16 of the symmetrical CAP binding site that prevent the binding. All three mutations affect the same amino-acid of CAP, glutamic acid 181. It is proposed that it is this amino-acid of CAP that makes contacts with base pairs 7 and 16 of the symmetrical recognition site. The authors deduced the specific chemical interactions by which amino-acid side chains at position 181 interact with base pairs 7 and 16 and the precise alignment between structures of the CAP and DNA in the intramolecular CAP–DNA complex. Several predictions can be drawn from this model, that we are currently testing, by site directed mutagenesis of the *crp* gene coding for CAP.

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Probing the catalytic mechanism of glyceraldehyde-3-phosphate dehydrogenase by site-specific mutagenesis

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The glycolytic glyceraldehyde-3-phosphate dehydrogenase (GAPDH) is a tetrameric enzyme, with cooperative properties, requiring NAD^+ as cofactor. GAPDH catalyses the oxidative phosphorylation of D-glyceraldehyde-3-phosphate into 1,3-bisphosphoglycerate, through a series of steps, including several chemical intermediates[1]. The 3D structure at high resolution determined by X-ray crystallography is now available for the lobster muscle enzyme and for the *B.stearothermophilus* enzyme[2,3]. Kinetic, chemical and crystallographic studies have enabled the proposal of a plausible molecular model which could account for catalysis and explain substrate specificity. In particular, amino-acids which could play a role in the catalytic mechanism have been identified. This includes the binding of coenzyme NAD^+ in which the adenine moiety binds to Asp 32 and the nicotinamide moiety binds to Asn 313 and Tyr 317. The substrate 3-phosphate binds to Thr 179, Arg 231, and Arg 195. The inorganic phsophate binds to Ser 148, Thr 150 and Thr 208. The Cys 149 residue is implicated in the formation of the acyl intermediate and His 176 is implicated in acid–base catalysis. Recently, two fragments of 1500 and 4000 nucleotides in length, inserted in pBR 322 and containing the structural GAPDH genes of *E.coli* and *B.stearothermophilus*, respectively, have been selected by complementation of an *E.coli* GAPDH mutant[4]. A very high efficiency of expression of the two cloned genes was observed in *E.coli*. The sequence of the two inserts has been determined[5,6]. In order to confirm the catalytic mechanism of GAPDH, different mutants were generated, by site-specific mutagenesis. We have replaced the histidine 176 at the active site with an aspartine residue. We also obtained a double mutant