not contribute significantly to the stability of the latent structure. Apparently the extended loop is driven into this taut conformation by the formation of strand 4A.

Latent PAI-1 is inactive presumably because part of its reactive centre loop is inaccessible or does not have the conformation to bind its cognate proteases. The residues expected to interact with protease[15], Ala 357–Glu 362 (P2–P4′), all reside in the extended loop on the surface of the molecule. The conformation of this loop is quite different from the arched loops formed by the reactive centres of other serine protease inhibitors[15]. Furthermore, Ala 357 (P2) and Glu 362 (P4′) would be expected to interact with tissue plasminogen activator (tPA) in the tPA–PAI-1 complex[15–18] but are partially buried in latent PAI-1.

Although the structure is not known of active PAI-1 or of any other active serpin, the structure of the intact, non-inhibitory serpin homologue ovalbumin has been reported[11]. Ovalbumin lacks strand 4A, and contains strand 1C in a conformation similar to that found in α_1-PI. The remainder of the reactive centre loop is folded into a surface helix (Fig. 3*b*). Active serpins may resemble ovalbumin in having strand 1C but, unlike ovalbumin, they probably contain a short, partially inserted strand 4A (refs 19–21). In ovalbumin, Arg 345 (P14) may prevent insertion of strand 4A (refs 11, 12). In other serpins, it is likely that the complete insertion of exposed reactive centre loop residues into strand 4A, either spontaneously (latent PAI-1) or upon cleavage (α_1-PI), drives the conformational change underlying inactivation.

PAI-1 is the only serpin known to undergo a transition to the latent, inactive conformation without cleavage under physiological conditions[14]. The transition may be facile because there are few stereochemical barriers to the insertion of strand 4A. Mutagenesis has shown that the sequence of the reactive centre loop itself (between P16–P2′) is not critical[22]. Therefore, strand insertion may be hindered by residues outside the reactive centre loop in other serpins but not in PAI-1. For example, residues Asn 49, Asp 177, Ser 330, Pro 391 and Gln 393 in α_1-PI occupy the same space held by residues in the extended loop in latent PAI-1 (assessed by superposition of latent PAI-1 and α_1-PI). The transition to the latent form is prevented *in vivo* by the binding to vitronectin in plasma[3,23].

It has been proposed that active serpins resemble ovalbumin[11]. To make the transition from an ovalbumin-like structure to one resembling latent PAI-1, residues in the β ribbon 3C–4C (coloured red in Fig. 3*b*) would have to be displaced. Indeed, these residues (dashed in Fig. 2*a*) are disordered in latent PAI-1. Further, interactions among a cluster of hydrophobic residues (Phe 208, Pro 289, Pro 369, Phe 370 and Pro 391) which are invariant throughout the protein family tether this β ribbon to the rest of the molecule in other serpin structures[7], but the interactions are absent in latent PAI-1. If active PAI-1 resembles ovalbumin in having a reactive centre loop positioned above sheet C (Fig. 3*b*), weakened interactions between the 3C–4C β ribbon and the rest of the protein could allow the collapse of the reactive centre loop to the position below sheet C seen in latent PAI-1.

The structural similarity of latent and cleaved PAI-1 was established by antibody cross-reactivity[13], and was surmised from the similar thermostabilities of cleaved serpins[5,24] and latent PAI-1 (refs 13, 14). Thermostability is likely to stem from insertion of strand 4A into sheet A, a structural feature common to the latent and cleaved molecules. Carrell *et al.*[14] have demonstrated that other serpins can adopt a thermostable inactive state on incubation in denaturants at low concentration. We propose that these thermostable forms may closely resemble latent PAI-1 with respect to full insertion of strand 4A and the position and conformation of the extended surface loop. □

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MutT protein specifically hydrolyses a potent mutagenic substrate for DNA synthesis

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ERRORS in the replication of DNA are a major source of spontaneous mutations, and a number of cellular functions are involved in correction of these errors to keep the frequency of spontaneous mutations very low[1]. We report here a novel mechanism which prevents replicational errors by degrading a potent mutagenic substrate for DNA synthesis. This error-avoiding process is catalysed by a protein encoded by the *mutT* gene of *Escherichia coli*, mutations of which increase the occurrence of A · T → C · G transversions 100 to 10,000 times the level of the wild type[2]. Spontaneous oxidation of dGTP forms 8-oxo-7,8-dihydro-2′-dGTP (8-oxodGTP), which is inserted opposite dA and dC residues of template DNA with almost equal efficiency, and the MutT protein specifically degrades 8-oxodGTP to the monophosphate. This indicates that elimination from the nucleotide pool of the oxidized form of guanine nucleotide is important for the high fidelity of DNA synthesis.

We have shown that a small amount of labelled dGMP was incorporated onto poly(dA)/oligo(dT)$_{20}$ template by the action of *E. coli* DNA polymerase III and the misincorporated nucleotide was not eliminated by the editing function of the polymerase[3]. But, the misincorporation was specifically prevented *in vitro* by the MutT protein which hydrolyses nucleoside triphosphates with an apparent preference for dGTP[3,4]. As the suppression of misincorporation occurred when a large amount of dGTP was present in the *in vitro* DNA synthesis system, we considered that the MutT protein might selectively degrade a mutagenic form of dGTP to prevent the dG·dA mispairing[3]. When dNMPs, prepared by P1 nuclease digestion from the DNA containing the misincorporated nucleotide, were subjected to

TABLE 1 Incorporation of 8-oxodGMP opposite dA and dC residues of template DNA

Template	Substrate	K_m (μM)	V_{max}	V_{max}/K_m (M^{-1})	Relative efficiency
dA	dTTP	1.0	2.0	2.0×10^{-6}	1.0
dA	8-oxodGTP	11	0.85	7.7×10^{-8}	0.039
dC	dGTP	1.0	2.1	2.1×10^{-6}	1.0
dC	8-oxodGTP	5.3	0.32	6.0×10^{-8}	0.029

The relative velocity for incorporation of nucleoside monophosphate opposite a single residue of dA or dC of each template DNA was determined as described[16]. The data were collected from experiments with various concentrations of substrate nucleotides (0.5–5.0 μM for dTTP and dGTP, and 5.0–50 μM for 8-oxodGTP). Intensities of bands in autoradiograms were measured using a Fujix 2000 Bio Image Analyzer. K_m and relative V_{max} were obtained from Lineweaver–Burk plots[17] of the data. Relative efficiency was calculated by dividing the V_{max}/K_m for incorporation of 8-oxodGMP by that of a correct substrate into each template DNA.

TABLE 2 Substrate specificity of MutT nucleoside triphosphatase

Substrate	K_m (μM)	V_{max}	V_{max}/K_m
8-oxodGTP	0.48	4.2	8,800
dGTP	1,100	4.8	4.3
dTTP	1,800	1.3	0.72
dCTP	1,700	0.28	0.16
GTP	1,200	1.6	1.4

Rates of hydrolysis of nucleoside triphosphates were determined by time-course experiments with eight different concentrations of the substrates (0.05–4.1 μM for 8-oxodGTP and 0.2–4.0 mM for other nucleotides). [α-^{32}P]dNTPs and -GTP were from Amersham. Unlabelled nucleotides were FPLC grade (Pharmacia-LKB), except for 8-oxodGTP. Hydrolysis of nucleoside triphosphates by the MutT protein was as described in the legend to Fig. 2. Amounts of the products were measured using a Fujix 2000 Bio Image Analyzer. Knowing the relative molecular mass of MutT protein[18] (15,000) and assuming that the preparation of protein was fully active, the turnover number (molecules of nucleoside monophosphate produced by one molecule of MutT protein in a second) was calculated. From Lineweaver–Burk plots[17] of the data, K_m and V_{max} for hydrolysis of each nucleoside triphosphate were obtained.

thin-layer chromatography, all the radioactivity associated with the misincorporated nucleotide migrated slower than the authentic dGMP (H.M., M. Akiyama, J.-Y. Mo and M.S., unpublished data). This meant that the misincorporated nucleotide is not dGMP but rather its derivative which is more negatively charged than dGMP. As deoxynucleotide carrying 8-oxoG has such properties[5,6], we suspected that 8-oxodGTP, present as a minor component in the labelled dGTP preparation that we used, may be inserted opposite dA residues of the template. The observation that dCMP and dAMP were selectively and equally incorporated opposite the 8-oxodG residue of a synthetic DNA template[7] also supported this view. All these findings prompted us to investigate whether 8-oxodGMP can be incorporated into DNA.

Using the α subunit of *E. coli* DNA polymerase III, which has DNA polymerizing capacity but not 3′→5′ exonuclease activity[8], we examined the incorporation of 8-oxodGMP onto synthetic DNA templates (Fig. 1). 8-oxodGTP was efficiently inserted opposite the dA residue as well as dC on the template.

There was no evidence for incorporation of 8-oxodGMP opposite dG and dT (data not shown). The Michaelis constant (K_m) and the maximum velocity (V_{max}) of each reaction are shown in Table 1. The apparent K_m for incorporation of 8-oxodGMP opposite dA and dC is only 5 to 10 times greater than those for the normal pairing (dTMP opposite dA and dGMP opposite dC). Relative efficiencies of incorporation of 8-oxodGMP opposite dA and dC were almost the same, and these values were 100- to 1,000-fold higher than those for misincorporation of usual nucleotides by the α subunit (misincorporation of dCMP or dAMP opposite dA and that of dGMP opposite dT)[9]. Note that misincorporation of dGMP opposite dA residue was not evident when a highly purified preparation of dGTP was used for the reaction (Fig. 1*a*). Thus, it is likely that previous observations[3,9] of *in vitro* misincorporation of

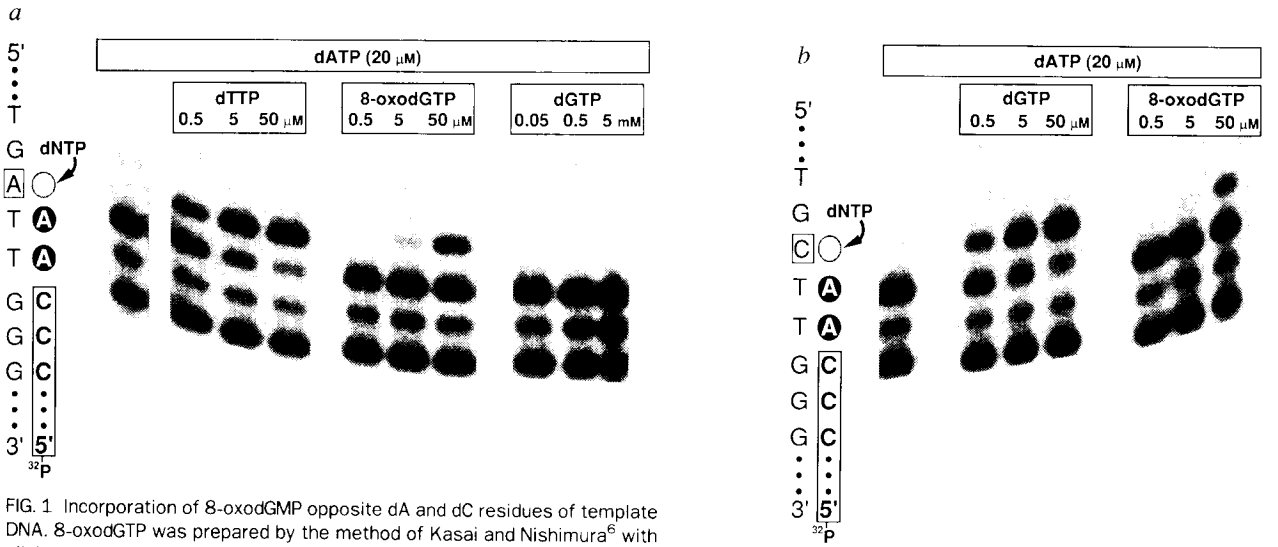

FIG. 1 Incorporation of 8-oxodGMP opposite dA and dC residues of template DNA. 8-oxodGTP was prepared by the method of Kasai and Nishimura[6] with slight modification and purified by two successive column chromatography steps with Spherisorb SAX and DEAE-MemSep (details to be published elsewhere). Purity and quantity of the preparation were examined in electrochemical measurements and ultraviolet spectrum analyses. dATP, dTTP and dGTP were from Pharmacia-LKB (FPLC-pure grade). Oligodeoxynucleotides were prepared by an automated DNA synthesizer (ABI model 381A) and purified by reversed-phase column chromatography. dA- and dC-template DNAs (3′-CGTCGGTTTTGCAGGGTT$^{A}_{C}$GTTAGTATG-5′) differ only in the tenth nucleotide from their 5′-termini. 5′-end labelling of primer DNA (5′-GCAGCCAAAACGTCCC-3′) and annealing of the primer to template DNAs were as described[9]. DNA synthesis on the dA-template was done with 1.4 units of highly purified preparation of α subunit[8] at 30 °C for 10 min. Reaction mixtures contained 20 μM dATP and varying concentrations of dTTP, 8-oxodGTP or dGTP. Other procedures were as described[9]. Autoradiograms were obtained using a Fujix 2000 Bio Image Analyzer (Fuji). *a*, Gel autoradiogram showing the incorporation of 8-oxodGMP opposite dA residues of template DNA. *b*, Gel autoradiogram showing the incorporation of 8-oxodGMP opposite dC residues of template DNA.

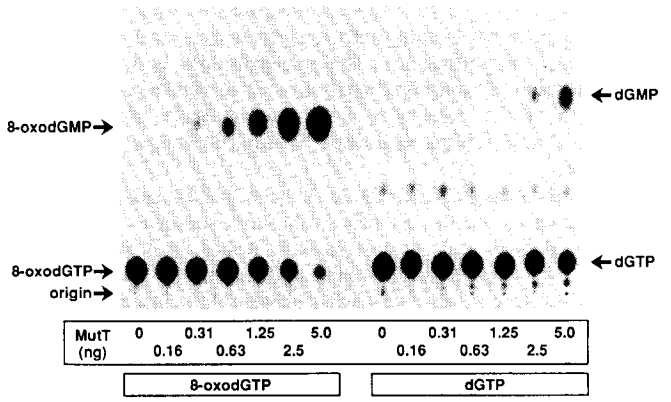

FIG. 2 Hydrolysis of 8-oxodGTP and dGTP by MutT protein. [α-^{32}P]8-oxodGTP was prepared from [α-^{32}P]dGTP (Amersham) and purified. [α-^{32}P]dGTP from Amersham was diluted with unlabelled dGTP (FPLC-pure grade, Pharmacia-LKB) and used without further purification. Protein concentration of a homogeneous preparation of MutT protein[3] was determined by the method of Bradford[19] with BSA as a standard. Hydrolysis reactions were at 30 °C for 10 min with varying amounts of MutT protein. The reaction mixture (12.5 μl) contained 40 μM 8-oxodGTP or dGTP, and other conditions were as described[3]. The production of 8-oxodGMP or dGMP was followed by thin-layer chromatography (PEI-cellulose plate, Merck) with 1 M LiCl and the autoradiogram was processed using a Fujix 2000 Bio Image Analyzer. Reaction mixtures (1 μl) were spotted on the same plate.

dGMP opposite dA were due to a minute amount of 8-oxodGTP present in the crude preparations of dGTP.

To examine whether the MutT protein efficiently hydrolyses 8-oxodGTP, we titrated the protein in a nucleoside triphosphatase assay with 40 μM 8-oxodGTP or dGTP and analysed the production of nucleoside monophosphates by thin-layer chromatography (Fig. 2). 8-oxodGTP was degraded 10 times faster than dGTP under the conditions used. This seemed to be due to a strong affinity of the protein to 8-oxodGTP. The kinetic parameters for hydrolysis of several nucleoside triphosphates by the MutT protein were measured (Table 2). The apparent K_m for the hydrolysis of 8-oxodGTP was 2,000 times lower than that of dGTP, whereas the V_{max} for both nucleotides was the same. The MutT protein hydrolysed other dNTPs and GTP with lower V_{max} and extremely high K_m values. Thus we can conclude that 8-oxodGTP is a specific substrate for the MutT protein.

8-OxodG was first described as a major lesion of DNA after exposure to reducing agents[5]. This lesion is caused by active oxygen species produced by many naturally occurring compounds, including cellular metabolic intermediates[6], as well as X-ray irradiation[10]. 8-oxodG is biologically important because a G·C→T·A transversion was induced at the site when DNA containing the damaged guanine was introduced into *E. coli* cells[11,12]. Results consistent with this were obtained in an *in vitro* DNA synthesis study using a synthetic DNA template containing 8-oxodG (ref. 7). An enzyme that specifically removes 8-oxoguanine from DNA has been detected in *E. coli*[13]. As inactivation of this enzyme by *mutM* (*fpg*) mutation leads to a 10-fold increase of G·C→T·A transversion frequency[14,15], the oxidation of the C-8 position of guanine residues of DNA would seem to occur spontaneously in *E. coli* cells.

As the oxidation of guanine proceeds *in vitro* more rapidly in dGTP than in DNA (unpublished data), it is conceivable that the oxidative damage to the base would occur more frequently in the nucleotide pool of *E. coli* cells than in their chromosomal DNA. From the distinct mutator action of *mutT*, we propose that the MutT protein has a major role in preventing the A · T→C · G transversion caused by 8-oxodGTP, formed spontaneously in the nucleotide pool. To protect genetic information from oxidation damage, cells seem to have at least two mechanisms, one functioning at substrate level (for the MutT protein) and the other at the DNA level (for the MutM (Fpg) protein).□

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Identification of a Fab interaction footprint site on an icosahedral virus by cryoelectron microscopy and X-ray crystallography

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BIOLOGICAL processes frequently require the formation of multiprotein or nucleoprotein complexes. Some of these complexes have been produced in homogeneous form, crystallized, and analysed at high resolution by X-ray crystallography (for example, see refs 1–3). Most, however, are too large or too unstable to crystallize. Individual components of such complexes can often be purified and analysed by crystallography. Here we report how the coordinated application of cryoelectron microscopy, three-dimensional image reconstruction, and X-ray crystallography provides a powerful approach to study large, unstable macromolecular complexes. Three-dimensional reconstructions of native cowpea mosaic virus (CMPV) and a complex of CPMV saturated with a Fab fragment of a monoclonal antibody against the virus have been determined at 23 Å resolution from low-irradiation images of unstained, frozen-hydrated samples. Despite the nominal resolution of the complex, the physical footprint of the Fab on the capsid surface and the orientation and position of the Fab have been determined to within a few ångstroms by fitting atomic models of CPMV[4] and Fab (Kol)[5] to reconstructed density maps.

Comoviruses are plant viruses whose structural and biological properties are strikingly similar to the animal picornaviruses[6,7]. For example, the protein capsids of CPMV, the type member of the comovirus group, and poliovirus, the most extensively studied picornavirus, are comparable (Fig. 1*a*). The CPMV coat contains 60 copies each of large (relative molecular mass 42,000 (M_r 42K) and small (24K) protein subunits arranged with icosahedral symmetry. Each large subunit (L) is composed of two antiparallel β-barrel domains which are positioned close to the icosahedral 3-fold axes. The β-barrel domain at the

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