TSE agent strains and PrP: reconciling structure and function

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The molecular structures of the infectious agents that cause transmissible spongiform encephalopathy (TSE) diseases are still not known despite the current wide acceptance of the prion hypothesis as the basis for their resolution. Here, data supporting and challenging the prion hypothesis in relation to both the biochemical and biological properties of TSE infectious agents are discussed. The need for the independent transmission of TSE agent-specific genetic information is described and the requirements for the molecule to carry this information are proposed. Such a molecule is likely to be a small nucleic acid encoding information to determine the diversity of the pathogenesis of TSE agents.

The prion hypothesis [1] is now 20 years old. In its original protein-only version, which is the one generally accepted when the term ‘prion’ or ‘prion hypothesis’ is used, protein was proposed as the sole component of the infectious agent causing the transmissible spongiform encephalopathies (TSEs), including scrapie in sheep, bovine spongiform encephalopathy (BSE) and Creutzfeldt–Jakob disease (CJD). However, despite stimulation from the prion and other hypotheses [2,3], the physicochemical and biological properties of the TSE infectious agent remain to be reconciled with the structure and biochemical properties of PrP. The nature of the causal agent continues to be the subject of debate.

The prion hypothesis was proposed because of the failure to identify a TSE-specific nucleic acid or to demonstrate its presence. Soon after publication of the prion hypothesis, the PrP protein was discovered [4]. Evidence accrued showing that PrP and TSE infectivity co-purified [5], and it was suggested that PrP was the essential component of the infectious agent – i.e. that TSE infections were caused by an infectious protein, PrP [6].

In 1985, the DNA encoding PrP was sequenced and it emerged that PrP is a host glycoprotein [7]. The normal form of the protein, PrP* and this has not been biochemical or characterized, nor have any differences in conformation been determined that, as predicted by the prion hypothesis, would render PrP*, but not the bulk of abnormal PrP, infectious. PrP*, the infectious protein, remains a hypothetical entity.

According to the prion hypothesis, it has been predicted that PrPSc should be able to catalyse the conversion of normal PrP into the abnormal form. Change is initiated spontaneously by mutated PrP, which is more susceptible to conformational change, or exogenously by PrP that is already in the abnormal form.

Despite its widespread acceptance, the prion hypothesis has yet to be reconciled with several properties of TSE agents, most notably the diversity in biological and biochemical properties of different TSE strains (Box 1). Here, a simpler explanation is sought by re-examining the case for a host-independent nucleic acid genome that carries the genetic information determining TSE strain properties and their diversity.

The relationship between PrP and infectivity

PrP in an abnormal form, PrPSc, is found as deposits in infected tissue as a pathological product of infection. TSE infectivity also tends to co-purify with PrPSc, but the role of the protein in the structure of the agent remains to be determined. Originally, PrP was distinguished from PrPSc operationally, by the solubility in detergents and sensitivity to protease digestion of PrPSc. In contrast to the sedimentibility and partial resistance to proteases of PrPSc, PrPSc has a higher degree of β-sheet than PrP and this has been correlated with structures predicted from NMR of recombinant PrP, suggesting that PrPSc has three α-helices and one small region of β-sheet [9].

Although there are high ratios of PrPSc per unit of TSE infectivity in some TSE models, in others it can be very difficult to detect PrPSc [10–12] and the ratios of PrPSc to infectivity might be lower. Some PrP in the PrPSc fraction can be separated from infectivity following detergent treatment [13–15]. In one case, nearly all detectable PrP was solubilized, but infectivity still sedimented [15]. Infectivity of a thermolabile TSE strain can be inactivated at relatively low temperatures, but resistance of PrPSc to protease digestion is not affected [16]. These data have led to the suggestion that only a subpopulation of PrP in the abnormal PrP fraction is infectious – i.e. the real PrPSc, alternatively named PrP* [17].

This structure has not been biochemically identified or characterized, nor have any differences in PrPSc, but not the PrPSc, infectious. PrP*, the infectious protein, remains a hypothetical entity.

According to the prion hypothesis, it has been predicted that PrPSc should be able to catalyse the conversion of normal PrP into the abnormal form, and this has been successfully performed in vitro [18]. A further prediction and a much greater test of the hypothesis is that the converted PrP should be infectious. No new infectivity has been detected to date [19,20].
Box 1. What are TSE strains?

As with all other infectious organisms, transmissible spongiform encephalopathy (TSE) agents from different sources exhibit a diversity of phenotypic properties due to the existence of different strains of TSE agents. The main challenge to the prion hypothesis has arisen from the necessity to explain this diversity. The multiplicity of biological properties is most readily demonstrated by transmitting TSE infections from different primary sources, such as a scrapie-infected sheep or cow infected with bovine spongiform encephalopathy (BSE), to inbred mice [a,b]. Although it is sometimes possible to distinguish between TSE isolates at primary passage into mice, it is necessary to passage the isolate several times through mice before the properties remain the same between passages. Most strikingly, incubation periods can vary dramatically in different inbred mouse strains, particularly those that differ in PrP genotype. In addition, mice that are heterozygous with respect to PrP genotype can have incubation periods intermediate between the incubation periods of the two homozygotes, or in some cases have incubation periods that are longer than either of the two parental strains, an effect called 'overdominance'.

The severity and distribution of neuropathological lesions also varies between strains. This is dramatically illustrated by the deposition of PrP in brains from mice infected with the ME7 or 87V TSE strains. In the ME7-infected brain PrP deposition is widespread, whereas in the 87V-infected brain PrP deposition is focal, localized, for example, to the CA2 region of the hippocampus and the thalamus [c]. There is no explanation at the molecular level for how TSE agents encode this precision of targeting or with which host mechanisms the agent interacts.

When scored semiquantitatively, pathological lesions can be used as a separate, independent tool to characterize strain phenotype. Classically, vacuolation is scored, although PrP deposition can also be used. A comparison of strains isolated from natural scrapie and BSE has recently been published [d]. PrPSc extracted from infected brain also has the potential for strain typing. Both the degree of glycosylation of PrPSc and the distribution of sizes of PrP fragments that resist protease digestion vary according to strain of agent and hence may also be of value for strain differentiation, although it should be noted that both types of modification to the protein structure are also influenced by host effects. Strains also vary in their resistance to inactivation with heat, which may have some future value for strain typing. Uniquely, heat-inactivation properties are intrinsic to the structure of the agent per se and are not dependent on interactions with the host.

Phenotypic properties of TSE agents can remain the same on serial passage, or they can change gradually or there can be a sudden switch in properties [e]. There are two possible explanations for these latter phenomena: either there are two or more extant strains of TSE agent present in the passage line, one of which is at a selective advantage (i.e. it replicates faster), or there is one strain present which mutates; mutants that replicate faster are then selected.

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The challenge from agent strain diversity: biophysical and biochemical properties

TSE agents are notoriously difficult to inactivate completely with heat. However, some of the factors that affect thermostability have now been identified. Although PrP genotype has little effect [21], TSE strains vary dramatically in their resistance to heat [16,21,22]. When heated in an aqueous environment, TSE infectivity exhibits an initial rapid reduction of titre, followed by a prolonged plateau during which little further inactivation takes place (Fig. 1a) [22,23]. When heated above physiological temperatures, infectivity is stable until an inactivation point temperature is reached. Above this point, there is a large decrease in titre. The inactivation point temperature varies with strain of agent (Fig. 1b) [16]. Heat inactivation of TSE infectivity, therefore, exhibits biphasic properties with respect to time of exposure and temperature. Similar effects are found with increasing pH (>pH 11) and the combination of high temperature and pH has a synergistic effect on TSE inactivation (R.A. Somerville, unpublished). These observations lead to the following conclusions. Biphasic kinetics fit model structure predictions in which the agent comprises two or more components. The variation in thermostability between TSE strains requires that one component differs in structure, probably covalently, and that this structure is dependent on the host. The survival of a subpopulation of infectivity is predicted to be induced by conversion of the agent into a more heat-resistant, protected form [16].

TSE infectivity becomes much more resistant to heat inactivation in dehydrating conditions [24]. Possibly the water of solvation is removed in a dry-heat environment and when the protected state is formed on heating in water [16]. Presumably the modified structure is more resistant to inactivation because the denaturation reaction cannot proceed.

TSE infectivity can be destroyed with some proteases at high concentrations and prolonged digestion times [5]. Destruction of infectivity correlated closely with the digestion of PrPSc in the TSE model tested [6]. These data are consistent with the hypothesis that the TSE agent has a protein component, probably PrP. Very high pH (>pH 14) may hydrolyse TSE infectivity, but detection of this phenomenon will be masked by the denaturation effect that occurs at lower pH (>pH 11) on hydrated TSE agent.

Exposure of TSE infectivity to sodium dodecyl sulphate (SDS, >1%) caused a reduction in titre that was dependent on the concentration of SDS and time of exposure [22]. Similarly, exposure to strong chaotropes (e.g. >3 M guanidine HCl) caused reductions in titre [25]. It is interesting to note that two TSE models that differed in both TSE strain and mouse strain varied in their sensitivity to SDS (Fig. 1c) [22]. Since the two mouse strains encode PrP with different amino acid sequences, either the
Opinion

Fig. 1. Differences in inactivation properties of TSE strains. (a) Effect of heating two strains of transmissible spongiform encephalopathy (TSE) at 126°C in an autoclave for different periods of time. Infectivity titre drops rapidly, after which little further inactivation takes place. (b) Effect of heating two TSE strains for 30 min at different temperatures. Little inactivation takes place at low temperatures, but above an inflection point (70°C for 22°C, 97°C for 22A) there is a marked reduction in surviving infectivity, which is temperature-dependent. (c) Effect of incubating two TSE strains from mice strains of different PrP genotypes in 5% sodium dodecyl sulphate for different times. There is a linear decrease in the amount of infectivity with time. The rate of loss of infectivity varies according to model and might be caused by either strain of agent, PrP genotype or a combination of both. For Figs 1a,c, original data [22] were fitted to biphasic curves using GraphPad Prism. Fig. 1b is from [16] and the curve is fitted to the equation derived therein. Reproduced, with permission, from [16,22].

A strain of agent or primary structure of PrP affects the stability of the agent with respect to detergent denaturation.

Inactivation with ionizing radiation has been used to study the size of biological structures, including the TSE agent. In calculations from first principles, it is assumed that a single hit destroys the radiation target and that the probability of hitting the target is proportional to its size. Hence the size (mass) of the target can be calculated from its residual activity. Radiation can determine the combined mass of the components required for a function within a more complex structure. For example, the target of some oligomeric enzymes is one of the monomers in some cases but the oligomer in other cases. In glycoproteins, the target is solely the polypeptide chain [26]. When viruses of known structure are subjected to radiation, there is a discrepancy between the target size calculated from first principles and the much larger actual size of nucleic acid that is presumed to be the target [27]. It is therefore a matter of debate as to the composition and size of the target for ionizing radiation when the structural components are not defined. TSE infectivity is more resistant to inactivation with ionizing radiation than any known conventional virus, although viruses with small genomes approach the resistance of the TSE agent [27]. Assuming the calculated size is independent of molecular composition, calculation of the size of the TSE agent from first principles gives \( M_r = 134,000 \), with a range of \( M_r = 64,000–150,000 \) [27]. This is equivalent to 400 nucleotides (nt) if the target is nucleic acid and five PrP (range 2–6) polypeptides if the target is PrP protein. If TSE agents are virus-like, genomic size could be up to tenfold greater than target size calculations. The overall range of probable sizes for a putative nucleic acid is therefore \( M_r = 64,000–1,600,000 \) (200–5000 nt). Since ionizing inactivation kinetics are monophasic with respect to dose, it is predicted that a single component of the agent's structure is targeted.

In summary, data from heating hydrated infectivity indicate an oligomeric structure in which water of solvation plays a role. It is unlikely that conformational variants of PrP would be sufficiently stable to preserve strain-specific conformations at near-denaturing temperatures. It is much more feasible that an agent-specific component forms one component of the structure, which is independent of the host, varies covalently in structure between TSE strains and is responsible for differences in thermostability. Further experiments comparing TSE models that vary in both TSE strain and PrP sequence may allow further differentiation of components of the agent that are agent-specific and any that are of host origin. For example, treatment with proteases and protein denaturants shows that protein is part of the structure. The difference between SDS inactivation of the two TSE models compared so far allows for either an agent-specific or a PrP-specific effect [22]. If the effect were controlled by PrP genotype, this would be direct biochemical evidence of a role for PrP in the structure of the infectious agent. The target for ionizing radiation is not known, but it is probably too large to be a single molecule of PrP. It could be either an oligomer of PrP or another, unidentified, component.

The challenge from agent strain diversity: biological properties

BSE has naturally and experimentally been passed through several hosts, but when its properties are characterized by serial passage in mice they are found to be very similar, despite differences in their passage history [28]. Thus, the phenotypic properties of the BSE strain of agent are retained despite transmission through a variety of different hosts that differ in PrP sequence. By contrast, some TSE strains show 'breakdown', whereby their phenotypic properties change stochastically on serial passage within a single strain of mouse [29]. This is interpreted as evidence of mutational change to the agent's genome, independently of the host and, in particular,
Box 2. The case against a nucleic genome: is it proven?

Whereas the smallest, discrete functional nucleic acids are the micro RNAs comprising ~21 nucleotides (nt), viroids have the smallest genomes, from ~250 nt. They may indicate a minimum size for putative transmissible spongiform encephalopathy (TSE) nucleic acids. This size range, or larger, is readily compatible with ionizing and UV radiation inactivation of TSE infectivity. TSE-infected tissue with a titre of 10^9 ID_50 g^{-1} would contain ~140 pg g^{-1} of a nucleic acid of 250 nt, assuming one molecule per infectious unit. Ideally, candidate TSE-specific nucleic acids are sought in high-titre, highly enriched infectivity extracts. However, these fractions contain large amounts of PrP^{Sc} and contaminants. Nucleases and chemical methods of nucleic acid destruction have little effect on TSE infectivity, whereas proteases and protein denaturants can destroy infectivity. Protein may protect a putative TSE nucleic acid, but removal of the protein presumably renders the naked nucleic acid non-infectious, preventing further monitoring by bioassay. TSE-specific nucleic acids could suffer several fates during extraction. The efficiency of recovery of very small amounts of nucleic acids of unknown structure from phenol-based extractions is unknown; some could segregate into a phenol phase. Protease digestion ultimately destroys TSE infectivity, but limited digestion has little effect, nor does sodium dodecyl sulphate (SDS) at low concentration or limited times. Much nucleic acid could remain associated with protein. Guanidine (+4 m) inactivates TSE infectivity effectively. Its use combined with prior extensive proteinase K digestion in SDS may be optimal for releasing the nucleic acid. Efficient concentration is then required.

In one series of experiments in which no candidate TSE nucleic acids were detected [a], nucleic acids contaminating the infectious fraction were destroyed with Zn^{2+} or nucleases before nucleic acid extraction. Samples were boiled in 2% SDS for 5 min, digested with proteinase K for 3 h then subjected to phenol-based extraction and ethanol precipitation. Analysis was performed by gel electrophoresis and silver staining.

An ingenious method for concentrating nucleic acids of differing sizes by reversing the direction of electrophoresis was used to enhance detection. However, despite the use of controls, without knowing the properties of TSE-specific nucleic acids it is impossible to estimate purification efficiency. Areas of concern include carryover of nucleic acid or Zn^{2+} to nucleic acid extractions, insufficient SDS–proteinase K digestion, losses into the phenol phase, damage to released nucleic acid by boiling and effectiveness of precipitation. Other have detected nucleic acids in purified infectivity extracts but none of them have been shown to be TSE-specific [b].

The ultimate challenge is to identify candidate TSE-specific nucleic acids, sequence them and test their specificity. A TSE-specific nucleic acid is likely to be small, present in small amounts and well protected, hence difficult to purify, its sequence is unknown and may have features hindering cloning and sequencing. Techniques for identifying and characterizing candidate sequences may require modification. Reverse transcription of RNA and amplification of minute amounts of small nucleic acid molecules is required before cloning and sequencing. Selective amplification may be needed. Evidence of TSE specificity can be sought by comparison with databases and established experimentally by Northern and polymerase chain reaction analysis. If candidate molecules are identified, the relationship between sequences from different TSE strains and their evolutionary history would be most informative.

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Box 3. Origins of PrPSc

The prion hypothesis requires the conversion of mature, normal PrPC into abnormally folded infectious PrPSc. This has led to the assumption that the conformational changes to PrP must occur either extracellularly or in endocytic pathways \[a\]. However, the glycosylation of PrPSc varies according to strain of transmissible spongiform encephalopathy (TSE) agent and is also affected by tissue type or area of brain \[b\]. If the differences between PrPC and PrPSc are due to posttranslational processing (folding) and are solely under the control of the TSE agent, other influences on PrPSc covalent structure will be minimal and should reflect that of PrPC. In these models, PrP must be synthesized normally, then either a subpopulation of PrPC must be selected to form PrPSc with a different degree of glycosylation to that of the donor PrPC, or there is selective TSE strain-specific and tissue-specific degradation of the carbohydrate moieties on PrPSc (Fig. I). Mechanisms for either process are unknown. If such mechanisms do not exist, differential glycosylation of PrPSc challenges the assumption that PrPSc is formed from mature PrPC.

If the prion hypothesis is invalid, it is possible to explain differences between PrPC and PrPSc by proposing that they arise during biosynthesis, that changes in post-translational processing are induced in infected cells, causing altered glycosylation \[b\] and abnormal folding of nascent PrP. If this is the case, both normal and abnormal forms of PrP are synthesized separately. The abnormal forms are susceptible to protease digestion at different sites near the N-terminus. Both the degree of glycosylation and the points of proteolytic cleavage are likely to be affected by strain of TSE agent, PrP genotype and host-tissue effects.

Fig. I. Putative pathways for control of PrPSc glycosylation.

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digestion? mRNASnase PPrPPrPSc precursorPrPSc Selection?

neurological disease and a TSE-like disease was transmitted from them. Since the new infection arose in unique circumstances it would not be surprising if the agent strain that had arisen de novo had different properties to those from other sources. Its incubation period after passing in hamsters was short, but otherwise the biological characteristics of the new TSE strain remain poorly characterized. These experiments have not been repeated in other laboratories, nor have similar results been obtained using other PrP mutations. However, the same mutation has been introduced by gene-targeting technology so that the only change to the mouse genome is one mutation of the PrP gene; no additional genes were added. No neurological disease has been detected in this model, nor has any TSE disease been transmitted from old, uninfected mice in which infectivity is most likely to be detected \[11,12\]. Transmission of a range of TSE sources to the mutated mice shows some with shorter incubation periods than controls, others longer. For example, the 263K TSE strain is more readily passed to the mutated mice than to controls and exhibits a shorter incubation period on second passage. Overdominance has also been observed with some TSE strains. In these experiments, changing the PrP sequence to mimic the mutant human form did not induce disease, at least within the lifespan of the mouse. Instead, the altered PrP acted as a new allele of the gene controlling incubation periods.

According to the prion hypothesis, PrP acts as a 'scaffold' on which a series of abnormal conformations are induced spontaneously or by interactions with PrP already in an abnormal form. The primary sequence of PrP plays a part in determining the overall properties of the agent since it affects conformation. Thus the genetic properties of the agent which ultimately determine its phenotypic expression are predicted to be a combination of the properties transmitted through conformation and the host genetics of PrP.

The alternative view is that all the genetic properties of the agent are encoded independently of the host by an informational molecule. In this model, PrP interacts directly during the replication of the informational molecule, specifically at the rate-limiting step in the replication pathway. Because of overdominance, PrP must act as a dimer or higher multimer \[39\]. Additionally, PrP may bind specifically to the informational molecule, providing the proteinaceous protection predicted by inactivation studies. Accordingly, although PrP may well be a structural component of the agent, its properties would not contribute to the genetic information of the agent.

Overall, the argument for an independent, mutatable TSE genome can be sustained, as can the argument for PrP acting to control replication for all the data discussed above. Indeed, the transgenic experiments neatly fulfil predictions from the replication site hypothesis \[3\]. Some predictions from the prion hypothesis have been less successful, or require much more research to test the claims made. Whether the issue can be satisfactorily resolved using experimental approaches similar to those described above remains an open question.

Predicting structure from function: what structure supports TSE agents' genetic information?

The hypotheses that evolved in the 1970s and crystallized into the virino hypothesis \[40,41\] continue to account for all the available data. The virino hypothesis proposes an oligomeric structure comprising an informational molecule that is...
independent of the host and protected by host protein, probably PrP as a dimer or higher multimer [39]. Originally, the structure of the informational molecule was not defined, although it was thought likely to be a nucleic acid. The hypothesis has fulfilled key predictions of the biological properties of TSE agent interaction with the host [3]. However, the prediction of an informational molecule has yet to be fulfilled, and for some this is sufficient reason for rejecting the hypothesis. If there is no independent informational molecule, then the hypothesis is indeed wrong. However, its properties may not have been sufficiently defined biochemically for a successful search to be conducted. If the informational molecule is not a conventional nucleic acid, its properties would be difficult to define from the present data. If the informational molecule is a nucleic acid, failure to find it might be because the definition of its properties are deficient, or the methodologies for hunting for it remain to be developed (Box 2). It is worth noting that small mammalian RNA species with previously unsuspected functions are still being discovered [42].

The informational molecule is probably a nucleic acid because it is the most biologically and biochemically conservative and orthodox structure to explain the diversity of properties of the TSE agent. A nucleic acid version of the virino hypothesis can now be refined to take account of data accumulated over many years, of mutability, intrinsic behaviour when heated, probable size range and the series of phenotypic properties cited above. The most likely candidate is predicted to be a small RNA because of its high apparent mutation rate in certain situations [43]. It is capable of carrying the information to specify incubation period, targeting of pathological lesions and the glycosylation of PrPSc (Box 3) – possibly through specific interactions that alter the normal function of PrP, other proteins or regulatory sequences of nucleic acids. Information is also required to induce its own replication (i.e. a polymerase-encoding site). The encoded information will be constrained by its physicochemical properties, probably through a high degree of secondary structure. It must interact directly with and be protected by PrP. It may exercise control, directly or indirectly, on the translation of PrP and/or on its post-translational modification (Box 3) [34].

Why is the debate about the causal agents of TSEs important? Identification of the means by which host-independent information is transmitted will lead to a much better understanding of TSE diseases. Identification of a host-independent informational molecule, if it is a nucleic acid, would certainly lead to a highly sensitive diagnostic test very quickly and most likely in the longer term to the development of therapeutic interventions.

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**p53 latency - out of the blind alley**

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A crucial mechanism by which p53 transcription function is regulated appears to operate through control of its DNA-binding properties. Allosteric/conformational and steric mechanisms of latency and activation of p53 DNA-binding function have been proposed. Both are inconsistent with recent data obtained by NMR and fluorescence correlation spectroscopy. This article presents the two-binding sites mechanism, which postulates that p53 is not a latent DNA-binding factor but is involved in multiple interactions with DNA via its core- and C-terminal domains, and that in the p53 tetramer-DNA complexes, binding of the C-terminal domain to DNA prevents interactions of the core domain with target DNA elements but not with nonspecific DNA sequences. We also discuss whether the conformations assumed by p53 when bound to structurally diverse targetDNAs are crucial determinants of interaction with co-activators and co-repressors of transcription.

The p53 protein is a sequence-specific transcription factor that plays a central role in cancer surveillance [1–4]. As a tumor suppressor, p53 controls cell-cycle progression, apoptosis and DNA repair following genotoxic stress and oncogene activation. Mutations in the p53 gene and/or defects in the p53-activating pathways appear to be general features of all cancer cells, and p53 loss or inactivation compromises genomic integrity. The p53 protein exerts its main functions by activating or repressing transcription of its target genes [1–4]. Protein products of several genes activated by p53 (such as p21, GADD45, Mdm2, Cyclin-G, IGF BP3, PIG3 and Bax) are required to regulate cell-cycle progression or apoptosis.

The p53 protein activates transcription by binding to DNA in a sequence-specific manner. Its binding site consists of two half-site decamers 5’-PuPuPuPuC(A/T)/PuPuPuPuPyPyPy-3’ (where Pu represents purine and Py represents pyrimidine) linked by a 0–13 nucleotide spacer. Each half-site comprises two copies of the pentamer sequence PuPuPuC(A/T) arranged head-to-head [1–3] and binds a dimer of p53, resulting in the formation of the functional p53-tetramer-DNA complex. The degenerate nature of the p53 DNA-binding sequences allows structural diversity of p53 DNA targets and might be crucial for the plasticity of responses to cellular signals.

The p53 protein has a modular structure and can be divided into three main domains (Fig. 1): the N-terminal transactivation domain (amino acids 1–99); the core domain (amino acids 100–300), which binds to specific DNA target sites; and the C-terminal domain (CTD; amino acids 301–393), which includes the tetramerization domain (amino acids 325–356) and a regulatory region (amino acids 363–393). The N-terminal domain consists of two contiguous transcriptional-activation subdomains (amino acids 1–42 and 43–63) and an adjacent proline-rich region (amino acids 62–91) that might be involved in the regulation of apoptosis [1–4]. The p53 protein can bind to DNA with high affinity when in a tetrameric complex, and the tetramerization domain permits the oligomerization of this protein. The unique property of p53 as a transcription factor is that it contains two DNA-binding sites. The p53 core domain, which is the target of most mutations in tumors, binds to specific DNA sequences. The second site is associated with the regulatory region in the CTD and binds with high affinity to single-stranded DNA (ssDNA) ends, insertion or deletion DNA mismatches, Holliday junctions, and damaged DNA. This second binding site has DNA-strand renaturation and DNA-strand-exchange activities [1,4].

In ‘normal’ unstressed cells, p53 is present at low levels or in a latent form [1–7]. Oncogene activation or exposure of cells to genotoxic agents results in two related processes. First, p53 is stabilized by disrupting p53-MDM2 interactions; MDM2 targets p53 for destruction via the ubiquitin–proteasome system [2,3]. Second, p53 is activated, leading to transactivation of p53-dependent gene transcription [1,3,5–7]. Both processes appear to be essential but can be clearly separated in different cellular settings as having different mechanisms and playing distinct roles in response to cellular stress. Thus, stabilization of p53 has been reported as being insufficient for induction of p53-dependent apoptosis and growth arrest [1–6]. Furthermore, there is a