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Unexpected structure of Epstein–Barr virus lytic cycle activator Zta

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Abstract

The key viral gene responsible for initiating the replicative cycle of Epstein–Barr virus (EBV), termed *BZLF1*, encodes the multifunctional protein Zta (ZEBRA or Z). It interacts with DNA as both a transcription and a replication factor, modulates both intracellular signal transduction and the DNA-damage response and manipulates cell cycle progression. Muller and colleagues have resolved the structure of Zta bound to DNA, which confirms some structural predictions but reveals an unexpected twist and a complex dimerization interface. Because EBV is associated with human disease, Zta presents a prime target for drug design.

EBV latency and the lytic replicative cycle

Epstein–Barr virus (EBV) is a member of the γ-herpes-virus family and is associated with a wide range of human diseases including nasopharyngeal carcinoma, Hodgkin's disease, Burkitt's lymphoma and lymphoproliferative diseases in immunosuppressed people [1]. Primary EBV infection in infancy is normally asymptomatic but leads to lifelong persistence of the virus in B-cells in a latent form, in which only a subset of EBV genes are expressed [2]. Primary infection in a young adult can lead to infectious mononucleosis and also results in lifelong persistence of the virus. In cancer cells, EBV is also present in a latent state. During latency, EBV is effectively hidden from the immune system but if viral replication is initiated and lytic replication ensues, the cells express EBV genes that are more readily recognized by the immune system. Therefore, the viral lytic cycle could be manipulated in two different therapeutic contexts: (i) activation of the lytic replicative cycle has been proposed as an approach to expose EBV cancer cells to the immune system and so kill them [3]; (ii) suppression of the early lytic replicative cycle might prevent the development of lymphoproliferative disease in immunosuppressed individuals, as documented in a model system [4]. Two EBV genes are central to the activation of the lytic replicative cycle, *BZLF1*, which encodes Zta (ZEBRA or *Z*), and *BRLF1*, which encodes Rta. Cellular signal transduction pathways induce the expression of these two transcription factors, which together activate the expression of other lytic cycle EBV genes. Both proteins are, therefore, suitable targets for the design of drugs that alter the function(s) of these proteins. The recent description of the structure of the DNA binding and dimerization regions of Zta by Muller

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and colleagues [5] has set the stage for further probing of the complex structural and functional properties of this transcription factor and also for future drug design.

A large body of work has revealed that Zta can be broadly divided into three regions: an N-terminal transactivation domain, a central DNA contact region and a dimerization region (reviewed in Refs [6-9]) (Figure 1a). The DNA binding region and dimerization region partly conform to the well-characterized bZIP (basic/leucine zipper) domain that is found in a family of cellular transcription factors such as fos/jun, C/EBPα and GCN4. Interestingly, Zta recognizes a wider range of DNA binding sites than other bZIP members. bZIP proteins are homo- or heterodimers that contain highly basic DNA binding regions adjacent to regions of α-helix that fold together as coiled coils (Figure 1b); the interaction with DNA is dependent on dimer formation [10-13]. Although the basic region and N-terminal part of the dimerization region of Zta display a high degree of homology with the bZIP consensus, the homology tails off, which raises doubts about the ability of the ZIP region of Zta to generate a strong dimerization interface [9]. Indeed, biophysical analyses of synthetic peptides corresponding to the ZIP part of the homodimerization region of Zta revealed it to be less stable than equivalent regions of canonical bZIP members [14]. Furthermore, DNA binding assays undertaken in stringent conditions revealed that the C-terminal part of the dimerization region, CT, is absolutely required for DNA binding function [15].

Dimer interface of Zta

The successful crystallization of the majority of the DNA and dimerization domain of Zta bound to DNA revealed that Zta only partly conforms to the bZIP structure, and revealed the unique contribution from the CT region [5]. A continuous stretch of α-helix that encompasses both the basic region and the ZIP region (to residue Met221) was identified, as expected for a bZIP protein (Figure 1b). However, an unexpected twist was observed at the end of the α-helix, which results in the orientation of the CT region back against the ZIP (Figure 1b). A further short region of α-helix and a longer stretch of amino acids from the CT region intercalate with the ZIP region, which results in intra- and intermolecular hydrophobic interactions between the CT and ZIP regions.

DNA binding by Zta

The high-resolution structure of Zta was derived from a version of Zta with a double mutation in the DNA contact region (Ser186Ala, Cys189Ser); unfortunately, the wild-type sequence generated only a low-resolution structure [5]. Both amino acid changes in the double mutation enable interaction with the AP1 site yet alter the DNA-binding specificity of Zta for other sites [16-19]. Through the use of molecular modelling, Muller and colleagues developed models to account for the altered DNA-binding specificity of mutations at Ser186. In the future, it will be interesting to see if this structure will also account for the recently identified altered DNA-binding specificity of the mutation at Cys189 [19]. Interestingly, analysis of the structure suggests a reason why Zta is able to interact with a broader range of DNA binding sites than other bZIP members: Zta forms more contacts with the phosphate backbone than, for example, fos/jun, which could make it less dependent on base-specific interactions [5].

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Concluding remarks and future perspectives

The definition of the structure of the DNA binding and dimerization domain of Zta sets the stage for molecular description of the many functions of Zta [5]. For example, Zta forms direct interactions with several cellular proteins [9] and, although point mutations that disrupt the interactions have been identified, the minimal regions of Zta required for the interactions have not been precisely defined. The structure of Zta will greatly aid the design of such future mapping experiments, and will also aid the mapping of the molecular mechanism by which Zta functions as a replication factor.

Zta manipulates cell cycle control in a cell-lineage-dependent manner. Cell cycle arrest with some S-phase-specific gene expression is observed in a variety of cell lines in response to Zta expression [20-22]. Cell cycle arrest is dependent on the basic region of Zta but, intriguingly, does not require DNA binding [23]. Therefore, it is possible that the DNA binding region of Zta has two independent roles: either to interact with specific DNA sequence elements or to interact with cellular proteins to promote cell cycle arrest. One route by which Zta can promote cell cycle arrest is through the upregulation of C/EBPa expression [24]. The revelation that amino acid residues required for interaction with C/EBPa are buried deep within the protein–DNA complex [5] suggests that the basic region of Zta might have two mutually exclusive binding partners: either DNA or C/EBPα. The resolution of this important question awaits further investigation.

The CT region is required for transcriptional activation by Zta [15] and it would be interesting to determine whether the CT region is simply required to aid dimer formation or whether it has additional functions. Unfortunately, the C-terminal half of the CT region was not amenable to crystallization [5] and so it is yet unknown whether the unresolved part of the CT region continues towards the basic region, further extending the interaction with the ZIP region, or whether it has a different structure. However, knowing the position of the first half of the CT region will help in the design of experiments to investigate this.

The most far-reaching implications of this work are for the design of novel antiviral agents. The ZIP region of Zta had previously been shown to be a potential target for antiviral agents [15] but the identification of the extended interface by Muller and colleagues [5] now provides a more extensive and high-resolution target. Specifically, the identification of a large hydrophobic pocket (Figure 1b) generated by the interaction of the ZIP and CT regions within the dimer should aid future drug design.

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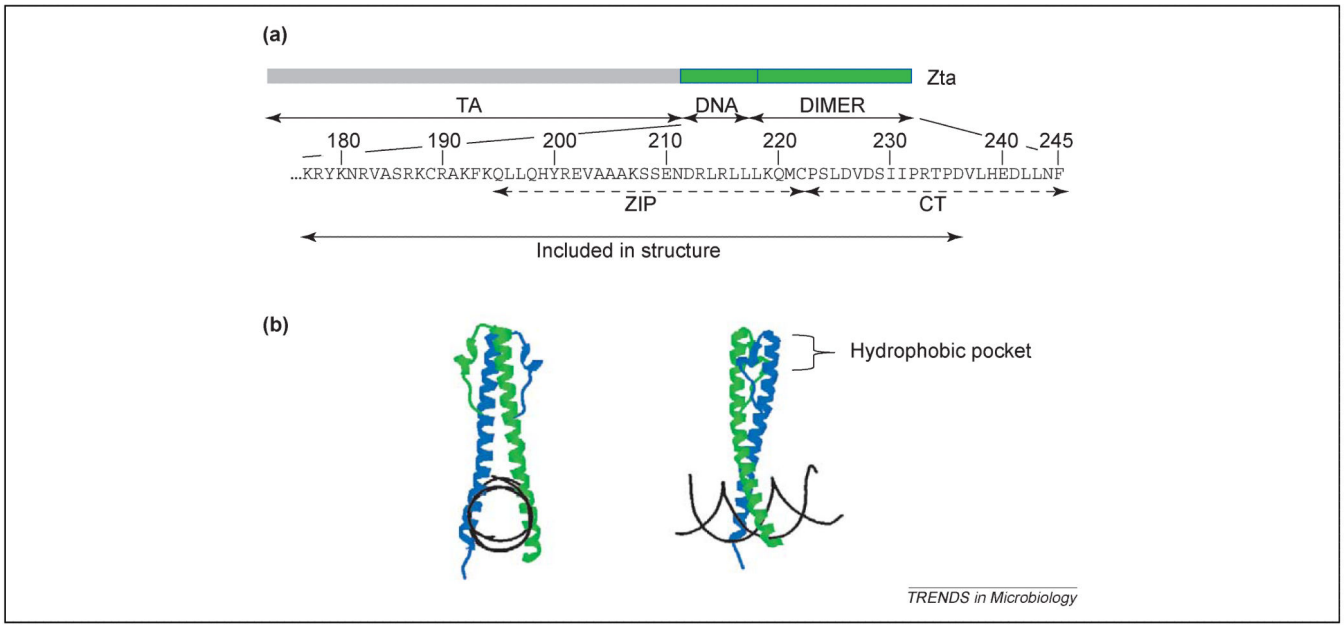

Figure 1. Schematic representation of the functional regions of Zta and its structure.

(a) The entire Zta protein is represented in the top panel. The transactivation (TA) domain is shown in grey and the DNA contact region and the dimerization region are shown in green and blue. The amino acid sequence of the DNA binding and dimerization domain is expanded below. The location of the region with homology to leucine zippers (ZIP) and the additional region required for dimerization function (CT) are indicated below the sequence. Beneath this, the residues of Zta included in the structure are indicated [5]. (b) The structure of Zta bound to DNA (generated from PDB 2C9L [5] using Protein Explorer [25]) is shown. One Zta molecule is coloured blue and the other green. The double-strand DNA helix is coloured black. A continuous α-helix extends through the basic and ZIP regions with the ZIP region coiled around itself. The left-hand orientation shows the turn and loop at the end of the zipper (residues 221–223). The CT region then turns back towards the DNA and forms interactions with both strands of the ZIP region. The right-hand orientation shows the complexity of the interaction of CT region with the ZIP region from the other monomer and also the location of the hydrophobic pocket. The location of the remainder of the CT region is not known.

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