Epstein–Barr virus: **LMP1 masquerades as an active receptor**

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The Epstein–Barr virus protein LMP1 is essential for transformation of resting B cells by the virus, but how it works is unclear. Recent results suggest that LMP1 acts as a constitutively active receptor that shares certain characteristics with members of the tumour necrosis factor receptor superfamily.

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Epstein–Barr virus (EBV) is a human γ-herpesvirus that is associated with several types of malignancy. EBV infects resting B cells and stimulates their proliferation via the coordinated expression of a number of 'latent cycle' genes, so-called because they are associated with latent virus infection. This process can be studied *in vitro*, where B cell infection leads to the outgrowth of virus-transformed lymphoblastoid cell lines expressing the nuclear antigens EBNA1, EBNA2, EBNA3A, EBNA3B, EBNA3C and EBNA-LP, and the latent membrane proteins LMP1, LMP2A and LMP2B. At least five of these proteins appear to be essential for growth transformation: the virus-genome-maintenance protein EBNA1, the transcriptional activators/regulators EBNA2, EBNA3A and EBNA3C, and the pleiotropic effector protein LMP1 [1].

LMP1 has attracted intense interest ever since it was found to induce the oncogenic transformation of rodent fibroblast cell lines. The multiple phenotypic effects of LMP1 expression have been most closely described, however, in human B lymphoma cell lines; they include cell aggregation, up-regulation of cell-surface markers such as CD40 and CD54, the activation of several cytokine genes, and the induction of cell-survival proteins such as Bcl2, BclxL, Mcl1 and A20. With hindsight, it is interesting to reflect how many of these changes recapitulate those induced in normal B cells by CD40 ligation. Now, as a spate of recent papers testify, the LMP1 and CD40 signalling pathways are becoming ever more closely intertwined.

Structurally, LMP1 is a 63 kDa phosphoprotein with a short (23 amino acid) amino-terminal sequence, six membrane-spanning domains (residues 24–186) and a long carboxy-terminal cytoplasmic tail (residues 187–386). LMP1 appears to oligomerise in the membrane, and the topology of two adjacent molecules is shown schematically in Figure 1. Genetic analysis has shown that both the transmembrane and carboxy-terminal regions are required for

LMP1-induced phenotypic changes in transfection assays, and for B-cell transformation by the virus itself. Interestingly, a recombinant virus expressing a truncated form of LMP1 with the amino terminus/transmembrane domain and only the 45 most membrane-proximal residues of the carboxy-terminal tail (residues 1–231) could induce B-cell growth for several weeks *in vitro*, although the infected cells remained fibroblast-dependent and proliferation eventually ceased [2]. This was the first suggestion that the LMP1 carboxyl terminus contained functionally distinct domains, a theme upon which the subsequent molecular dissection of LMP1 signalling pathways has elaborated.

LMP1 expression leads to the rapid activation of the transcription factor NFκB, and transient assays with deletion mutants identified two 'carboxy-terminal activating regions' that contributed to this effect — the membrane-proximal CTAR1 (residues 194–232) and the membrane-distal CTAR2 (residues 351–386) [3,4]. Although CTAR1 corresponded to what appeared to be the major effector domain for cell transformation, CTAR2 was the stronger NFκB-activating domain, accounting for 70–80% of the induction in a variety of cell types. Another more recent finding [5] links LMP1 to activation of c-Jun amino-terminal kinase (JNK, also known as stress-activated protein kinase, SAPK). JNK activation leads directly to induction of the transcription factor AP1, a dimer composed of members of the Jun or Fos family, which has been implicated in growth control in a number of contexts.

JNK activation by LMP1 occurs with kinetics that mirror NFκB activation [6]. When analysed by transient transfection in the human embryonic kidney cell line 293, this JNK/AP1 induction maps entirely to CTAR2 [5,6]. The results of recombinant virus experiments involving LMP1 mutants make it clear that CTAR2 has no B-cell growth transforming ability in the absence of the CTAR1 domain. However, the link between CTAR2 and transcription factor activation is consistent with this distal domain having an important auxiliary role in the transformation process. Certainly, in B-cell lines transformed with a mini-EBV construct carrying a tetracycline-conditional LMP1 gene, re-entry of cells into cycle upon re-induction of LMP1 expression correlates closely with the kinetics of JNK activation [5].

Against this background, what do we know about the molecular interactions through which CTAR1 and CTAR2 transmit their signals? The seminal paper of Mosialos and colleagues [7] first showed, using yeast two-hybrid analysis, that the carboxy-terminal half of LMP1 interacts with a

molecule of the tumour necrosis factor (TNF) receptor-associated factor (TRAF) family. This immediately drew a parallel between LMP1 and members of the TNF receptor superfamily, such as TNFRII and CD40, which also bind TRAFs via their intracytoplasmic carboxy-terminal domains. TRAF binding by LMP1 mapped within the CTAR1 region and involved a PXQXT motif (residues 204–208; where X can be any amino acid) that was also present in the carboxy-terminal sequences of CD40 and certain other TNF receptor family members.

LMP1 showed strongest interactions with TRAF3 and TRAF1, but was also capable of binding TRAF2 [8,9]. This was of particular interest, as TRAF2 is known to mediate NFκB activation by CD40 and TNFRII. An equivalent role for TRAF2 in LMP1 signal transduction was confirmed when it was found that CTAR1 induction of NFκB could be blocked by a dominant-negative mutant form of TRAF2 [8–10]. The signalling components that link CTAR1/TRAF2 to NFκB are currently unknown. By analogy with the events following TNFR activation, however, a TRAF2-bound kinase called NFκB-inducing kinase (NIK) may be involved. NIK activation leads to phosphorylation of IκB kinase (IKK), which in turn induces phosphorylation of IκBα and the subsequent release of functional NFκB (reviewed in [11] and illustrated in Figure 1). Furthermore, recent work has shown that CTAR1 binds another TRAF species, TRAF5, which can also activate NFκB [12].

Until recently, the molecular connections at CTAR2 have been a matter for speculation. No direct interaction was detectable between CTAR2 and any of the TRAF species. Interestingly, however, a dominant-negative mutant form of TRAF2 partially inhibited CTAR2-induced NFκB activation, as did other TRAF2-interacting proteins, such as TANK/I-TRAF and A20 [8,10]. These phenomena could now be explained by recent work in which yeast two-hybrid and biochemical analyses showed that CTAR2 binds to the TNFR-associated death domain (TRADD) protein [13]. TRADD is known by virtue of its interaction with TNFRI, where it acts as a platform for the recruitment of other proteins; one of these is TRAF2 and this interaction leads to both NFκB and JNK activation [14].

The above findings are most consistent with the view that the transmission of signals from CTAR2 occurs via a CTAR2–TRADD–TRAF2 complex. The signalling components downstream of this complex are not yet known, but, by analogy with TNFRI signalling pathways, it is possible that the NFκB and JNK activating signals bifurcate at the level of TRAF2. The NIK–IKK–IκB pathway could lead to NFκB activation, whereas the pathway leading to JNK may involve a recently described germinal centre kinase-related (GCKR) protein that is downstream of

Figure 1
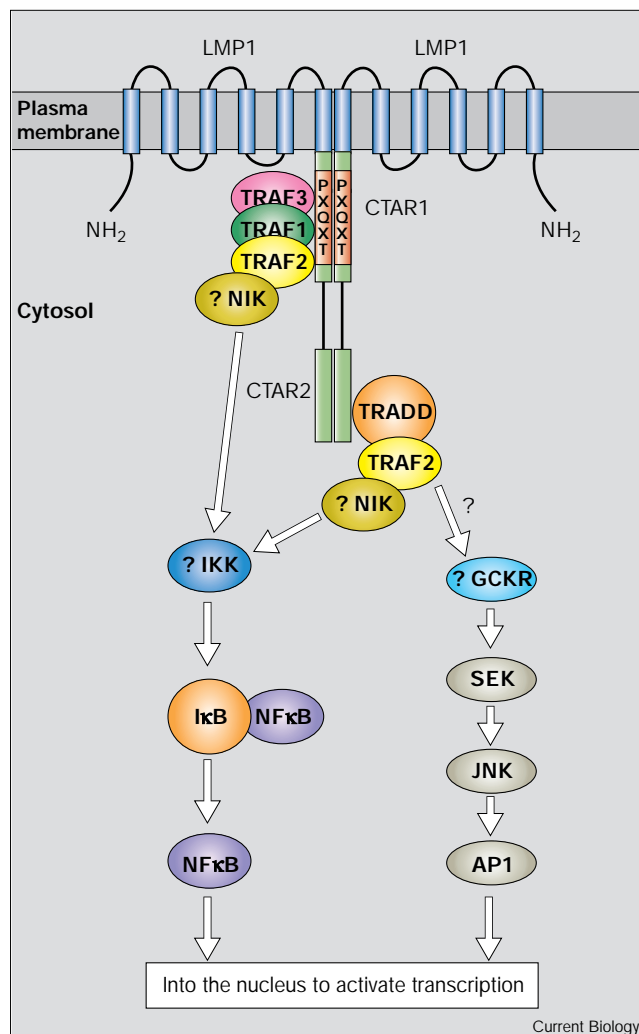
The pathways by which the EBV protein LMP1 is thought to signal inside cells. LMP1 has a short cytoplasmic amino terminus, six transmembrane domains (which confer aggregation at the cell membrane) and a long cytoplasmic carboxy-terminal tail containing two effector domains, CTAR1 and CTAR2. The CTAR1 domain, which is essential for EBV-induced B-cell transformation, binds TRAF1, TRAF2 and TRAF3; TRAF2 mediates NF-κB activation, presumably via the NIK–IKK–IκB pathway. The CTAR2 domain, which is important for long-term outgrowth of infected B cells, binds TRADD, and this is thought to recruit TRAF2, leading to both NFκB and JNK–AP1 activation through a bifurcating pathway; induction of NFκB may also occur via the NIK–IKK–IκB pathway, while the connection to SEK–JNK–AP1 activation may be via the recently described GCKR protein.

TRAF2 but upstream of SEK–JNK–AP1 (Figure 1) [15]. Bifurcation of the CTAR2 signalling pathway must occur because, as a recent study shows [6], blockade of NFκB activation by a constitutively active IκBα mutant does not affect the LMP1–JNK connection and, conversely, expression of a dominant-negative form of SEK blocks JNK but not NFκB activation.

A key distinction between LMP1 and the TNFR family members is that the activity of the viral protein is constitutive, rather than dependent upon the binding of a cognate ligand. The strong inference from early work on the distribution of LMP1 mutants within cells was that LMP1 function is dependent upon oligomerisation in the membrane, presumably via intermolecular associations of the six membrane-spanning domains. This has now been elegantly confirmed in experiments with chimeric molecules, in which the extracellular and transmembrane regions of CD2, CD4 or a member of the TNFR superfamily, NGFR, have been linked to the intracytoplasmic carboxyl terminus of LMP1 — LMP1-mediated signalling is now seen only following antibody-induced or ligand-induced aggregation of the chimaera [16,17]. Conversely, the carboxy-terminal tail of CD40 can be rendered constitutively active by linkage to the amino-terminal and hydrophobic domains of LMP1, and indeed this chimaera mimics many aspects of wild-type LMP1 function [6,18].

EBV, particularly its product LMP1, thus provides a telling example of how ancient viruses can usurp cellular pathways of central physiological importance for their own ends. The pursuit of LMP1 signalling will no doubt continue to provide an invaluable side entrance into the complex biology of the TNFR/CD40 family.

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