The c-Rel Transcription Factor in Development and Disease

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Abstract
c-Rel is a member of the nuclear factor κB (NF-κB) transcription factor family. Unlike other NF-κB proteins that are expressed in a variety of cell types, high levels of c-Rel expression are found primarily in B and T cells, with many c-Rel target genes involved in lymphoid cell growth and survival. In addition to c-Rel playing a major role in mammalian B and T cell function, the human c-rel gene (REL) is a susceptibility locus for certain autoimmune diseases such as arthritis, psoriasis, and celiac disease. The REL locus is also frequently altered (amplified, mutated, rearranged), and expression of REL is increased in a variety of B and T cell malignancies and, to a lesser extent, in other cancer types. Thus, agents that modulate REL activity may have therapeutic benefits for certain human cancers and chronic inflammatory diseases.

Keywords
c-Rel, REL, NF-κB, signal transduction, arthritis, cancer, B cells, T cells

The c-Rel transcription factor is a unique member of the vertebrate nuclear factor κB (NF-κB) family, primarily because of its pervasive and specific role in mammalian B and T cell differentiation and function, as well as in human disease. As such, c-Rel is of interest to many researchers and clinicians studying mammalian immunology and human immunological disorders, especially autoimmune diseases and hematopoietic cancers. This broad-based review describes the attributes of c-Rel structure, function, and regulation that confer it with its normal and pathological biological activities. In this review, c-Rel is used to refer generically to c-Rel proteins, whereas REL refers specifically to human c-Rel.

Structure of c-Rel and DNA-Binding Activity

Human REL is a 587–amino acid protein (Fig. 1). Like other mammalian NF-κB proteins (i.e., NFκB1/p50, NFκB2/p52, RelA/p65, RelB), REL contains a highly conserved N-terminal DNA-binding/dimerization domain called the REL homology domain (RHD).¹ The C-terminal half of REL is composed of sequences that affect its ability to activate transcription: That is, 2 C-terminal transactivation subdomains (TAD1 and 2)²-⁴ are separated from the RHD by a transactivation inhibitory domain (RID).⁵

REL has an optimal DNA-binding site preference for a target sequence that is slightly different from other NF-κB subunit homodimers. PCR-based site selection indicates that c-Rel has a broader target sequence recognition ability than p65 and p50, with 5’-NGGRN(A/T)TTCC-3’ identified as the optimal c-Rel binding sequence.⁶ The X-ray crystal structure of REL bound to a κB site (5’AGAAATTCC3’) from the IL-2 enhancer shows that c-Rel uses residues to bind DNA that are distinct from other NF-κB proteins, a property that may endow REL with its different target sequence preference. Interestingly, certain amino acids in these c-Rel-specific DNA-binding sequences are mutated in v-Rel,⁷ the viral oncogenic version of avian c-Rel. Mutagenesis studies also indicate that c-Rel has DNA-binding residues that are distinct from other NF-κB proteins.⁸ In most cells, REL exists either as a homodimer or a heterodimer with p50, but c-Rel can also form dimers with p65 and NFκB2.

Protein Modifications and Protein-Protein Interactions of c-Rel

A number of posttranslational modifications of c-Rel have been described. Within the RHD, c-Rel has been shown to undergo phosphorylation, acetylation, and, within the C-terminal sequences, phosphorylation and ubiquitination. In most cases, the functional relevance of

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these modifications is unclear. For example, PKA can phosphorylate a conserved site (R-R-X-S) in the c-Rel RHD domain, but whether and when such phosphorylation occurs in vivo is not known. Similarly, residues in the C-terminal half of c-Rel can be phosphorylated in vitro by IKKβ but have not been shown to be phosphorylated in vivo. Nevertheless, one of these IKK phosphorylation sites is mutated in some human B cell lymphomas. c-Rel has also been reported to be a substrate for NF-κB-inducing kinase (NIK) and a tyrosine kinase in myeloid cells following G-CSF stimulation. Although chicken c-Rel undergoes ubiquitin-mediated degradation due to modification of C-terminal lysines, the absence of lysine residues in the C-terminal half of REL may indicate that this is a species-specific mode of c-Rel regulation. Finally, a conserved Cys residue in a DNA-binding sequence of c-Rel allows for redox-sensitive DNA binding, where reduced REL binds DNA better than oxidized REL. The redox state of c-Rel also appears to affect its ability to be phosphorylated.

c-Rel is also subject to posttranscriptional processing. For example, alternative splicing can remove part of the central transactivation inhibitory domain RID, which increases both the DNA-binding and transactivating activity of REL; of note, this alternatively spliced form of REL mRNA is overexpressed in B lymphoma cell lines. c-Rel can be a substrate for caspase-mediated cleavage, which renders it inactive, a process that may modulate its role as an anti-apoptotic regulator.

Table 1. Non-NF-κB/IκB Proteins That Can Interact with c-Rel

<table>
<thead>
<tr>
<th>Protein</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Transcription factors</td>
<td></td>
</tr>
<tr>
<td>C/EBP/β</td>
<td>133</td>
</tr>
<tr>
<td>IκB</td>
<td>134</td>
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<tr>
<td>NFATc</td>
<td>23</td>
</tr>
<tr>
<td>Sp1</td>
<td>135</td>
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<tr>
<td>Foxp3</td>
<td>136</td>
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<tr>
<td>ΔNP63α</td>
<td>19</td>
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<tr>
<td>Androgen receptor</td>
<td>137</td>
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<tr>
<td>Estrogen receptor</td>
<td>138, 139</td>
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<tr>
<td>Transcription-mediating proteins</td>
<td></td>
</tr>
<tr>
<td>TBP</td>
<td>140</td>
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<tr>
<td>TFIIIB</td>
<td>140</td>
</tr>
<tr>
<td>p300/CBP</td>
<td>108</td>
</tr>
<tr>
<td>CAPERα</td>
<td>141</td>
</tr>
<tr>
<td>Cdk2/cyclinE</td>
<td>142</td>
</tr>
<tr>
<td>Aof1</td>
<td>143</td>
</tr>
<tr>
<td>Nuclear import</td>
<td></td>
</tr>
<tr>
<td>Importin alpha</td>
<td>144</td>
</tr>
<tr>
<td>Protein modifying/signaling</td>
<td></td>
</tr>
<tr>
<td>BAFF-R</td>
<td>145</td>
</tr>
<tr>
<td>CD40</td>
<td>20</td>
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<tr>
<td>Calmodulin</td>
<td>146, 147</td>
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<tr>
<td>Myotrophin/VI</td>
<td>148</td>
</tr>
<tr>
<td>Cot/Tpl2</td>
<td>149</td>
</tr>
<tr>
<td>ABIN2</td>
<td>149</td>
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<tr>
<td>PAPOLA</td>
<td>149</td>
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<tr>
<td>Erbin</td>
<td>150</td>
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<tr>
<td>JNK1</td>
<td>151</td>
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<tr>
<td>NIK</td>
<td>152</td>
</tr>
<tr>
<td>PKA-beta</td>
<td>153</td>
</tr>
<tr>
<td>Protein phosphatase X</td>
<td>154</td>
</tr>
<tr>
<td>Pin-1</td>
<td>155</td>
</tr>
<tr>
<td>Viral protein</td>
<td></td>
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<tr>
<td>HTLV-I Tax</td>
<td>156</td>
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</table>

Direct c-Rel Target Genes

Although there is considerable overlap in the DNA sequences and genes bound by the various NF-κB dimers, some genes do appear to be preferred targets for c-Rel, whether bound by c-Rel homodimers or by c-Rel heterodimers. Not surprisingly, based on the immune cell-specific functions of c-Rel (see below), many of these genes are involved in immune cell proliferation, survival (anti-apoptotic), or function (Table 2).

Computational modeling of sequences upstream of c-Rel target genes suggests that the c-Rel regulatory module consists of multiple closely clustered c-Rel binding sites. Furthermore, c-Rel has been shown to bind cooperatively at certain promoters with other transcription factors; for example, c-Rel interacts with NFATc1 on the CD154 gene promoter.

The C-terminal transactivation domain of c-Rel may have a specific role in regulating genes involved in B cell
Table 2. Partial List of c-Rel Target Genes

<table>
<thead>
<tr>
<th>Gene/Protein</th>
<th>Protein Function</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cell proliferation/cell growth</td>
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<tr>
<td>c-Rel</td>
<td>Transcription factor</td>
<td>157</td>
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<tr>
<td>c-Myc</td>
<td>Transcription factor</td>
<td>44, 158</td>
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<tr>
<td>IRF-4</td>
<td>Transcription factor</td>
<td>159</td>
</tr>
<tr>
<td>E2F3a</td>
<td>Transcription factor</td>
<td>57</td>
</tr>
<tr>
<td>EP300</td>
<td>Histone acetyltransferase</td>
<td>160</td>
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<tr>
<td>CD21</td>
<td>Complement receptor</td>
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<tr>
<td>CD40</td>
<td>Cell surface receptor</td>
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<tr>
<td>SFN</td>
<td>I-4-3-3 protein</td>
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<tr>
<td>GM-CSF</td>
<td>Hematopoietic growth factor</td>
<td>22, 162, 163</td>
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<tr>
<td>TGFβ</td>
<td>Growth factor</td>
<td>160</td>
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<tr>
<td>IL-2</td>
<td>Cytokine</td>
<td>22, 41, 163</td>
</tr>
<tr>
<td>IL-4</td>
<td>T cell cytokine</td>
<td>22, 164</td>
</tr>
<tr>
<td>Apoptosis/cell survival</td>
<td></td>
<td></td>
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<tr>
<td>Bcl-2</td>
<td>Anti-apoptotic</td>
<td>54, 165</td>
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<tr>
<td>Bfl-1/A1</td>
<td>Anti-apoptotic</td>
<td>60, 61</td>
</tr>
<tr>
<td>Bcl-XL</td>
<td>Anti-apoptotic</td>
<td>62</td>
</tr>
<tr>
<td>miR-21</td>
<td>Pro-apoptotic for β cells</td>
<td>228</td>
</tr>
<tr>
<td>Adhesion/cell architecture</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ICAM-1</td>
<td>Cell adhesion</td>
<td>166, 167</td>
</tr>
<tr>
<td>Selectin</td>
<td>Cell adhesion; binds sugars</td>
<td>160</td>
</tr>
<tr>
<td>MMP-1</td>
<td>Metalloproteinase</td>
<td>160</td>
</tr>
<tr>
<td>EPHB2</td>
<td>Receptor tyrosine kinase (repressed)</td>
<td>168</td>
</tr>
<tr>
<td>Immune cell function</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gamma1</td>
<td>Ig heavy chain</td>
<td>63</td>
</tr>
<tr>
<td>Gamma4</td>
<td>Ig heavy chain</td>
<td>64</td>
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<tr>
<td>TNF-α</td>
<td>Cytokine</td>
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<tr>
<td>IL-12</td>
<td>p35 cytokine</td>
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<tr>
<td>IL-13</td>
<td>Cytokine</td>
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<tr>
<td>IL-21</td>
<td>Cytokine</td>
<td>52</td>
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<tr>
<td>IL-23</td>
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<tr>
<td>CD40L</td>
<td>CD40 ligand</td>
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<tr>
<td>Blys/BAFF</td>
<td>TNF-like cytokine</td>
<td>171</td>
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<tr>
<td>IP-10</td>
<td>Chemokine</td>
<td>22</td>
</tr>
<tr>
<td>Ligi</td>
<td>GTPase</td>
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<tr>
<td>MIG</td>
<td>Macrophage cytokine</td>
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<tr>
<td>Foxp3</td>
<td>Transcription factor</td>
<td>172</td>
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<tr>
<td>DNA repair/damage</td>
<td></td>
<td></td>
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<tr>
<td>ATM</td>
<td>Protein kinase</td>
<td>160</td>
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<tr>
<td>Claspin</td>
<td>Cell cycle kinase</td>
<td>173</td>
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<tr>
<td>Skp2</td>
<td>S-phase kinase-associated factor</td>
<td>174</td>
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Table 3. Phenotypes of c-rel Mouse Knockouts

<table>
<thead>
<tr>
<th>Disrupted Genes</th>
<th>Phenotype</th>
<th>References</th>
</tr>
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<tbody>
<tr>
<td>c-rel</td>
<td>Reduced B cell proliferation, survival, and antibody expression</td>
<td>40</td>
</tr>
<tr>
<td></td>
<td>Reduction in induced arthritis</td>
<td>75</td>
</tr>
<tr>
<td></td>
<td>Sensitivity to Toxoplasma gondii</td>
<td>175</td>
</tr>
<tr>
<td></td>
<td>Increased sepsis</td>
<td>176</td>
</tr>
<tr>
<td></td>
<td>T cell defects</td>
<td>49, 177</td>
</tr>
<tr>
<td></td>
<td>Dendritic cell defects</td>
<td>178, 179</td>
</tr>
<tr>
<td></td>
<td>Reduction in induced diabetes</td>
<td>180</td>
</tr>
<tr>
<td></td>
<td>Reduction in induced colitis</td>
<td>181</td>
</tr>
<tr>
<td></td>
<td>Liver regeneration defect</td>
<td>182</td>
</tr>
<tr>
<td></td>
<td>Reduced synaptic plasticity and memory</td>
<td>183, 184</td>
</tr>
<tr>
<td>c-relΔCOOH</td>
<td>Increased B cell numbers in lymph nodes</td>
<td>185</td>
</tr>
<tr>
<td>c-rel/nfkb1</td>
<td>B cell developmental defects, TLR signals impaired</td>
<td>186</td>
</tr>
<tr>
<td></td>
<td>Impaired CD4+ T cell responses</td>
<td>50, 56, 179</td>
</tr>
<tr>
<td>c-rel/relA</td>
<td>Embryonic lethal, multiple hematopoietic defects</td>
<td>44, 187</td>
</tr>
<tr>
<td>c-rel/relA/tnf</td>
<td>Neonatal lethal, multiple epidermal defects</td>
<td>76</td>
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</table>

Role of c-Rel in Normal Immune Cell Function

c-Rel is expressed at the highest levels in a wide variety of hemopoietic cells. Nevertheless, studies using knockout mice have shown that c-Rel is generally not essential for normal hematopoiesis and lymphopoiesis. Instead, c-Rel is required for a number of specialized functions in mature T and B cells. A summary of the phenotypes reported in studies using c-rel knockout mice is presented in Table 3.

c-Rel plays a key role in the development of regulatory T cells. Several recent studies have shown that c-Rel is required for the development in the thymus of CD4 regulatory T cells (Tregs). Tregs, which consist of a subset of T lymphocytes that express the lineage-specific transcription factor Foxp3, are required to suppress the activity of autoreactive T cells that escape negative selection and limit the duration and strength of normal T cell responses. In c-rel knockout mice (c-rel-/- mice), only ~15% of normal Treg numbers develop in the thymus. The reduced Treg population in c-rel-/- mice is due to a thymocyte-intrinsic defect that reflects the high level of c-Rel expression in

proliferation. For example, the addition of the v-Rel transactivation domain onto the RelA RHD creates a transforming protein in avian lymphoid cells, but the addition of the RelA transactivation domain onto the c-Rel RHD does not. Target genes of c-Rel that are involved in various developmental or disease processes are described in relevant sections of the text below.
Tregs. The second step in Treg development, the induction of foxp3 transcription, is dependent on common γ-chain cytokine signals. Emerging evidence indicates that c-Rel directly controls the induction of foxp3 transcription during Treg differentiation. Within the foxp3 locus, there are c-Rel binding sites in the promoter, in a conserved element (CNS2) essential for stable foxp3 transcription, and in the CNS3 element, which like c-Rel is required for the development of the majority of Foxp3-positive Tregs in the thymus. The finding that Foxp3 expression in the residual c-rel−/− Tregs is normal suggests that c-Rel does not play a direct role in controlling the maintenance of foxp3 transcription but instead that c-Rel is required to establish a chromatin structure within the foxp3 gene that allows other transcription factors access to the locus. Given that c-Rel is not activated by the common γ-chain cytokine signals required for the induction of Foxp3 expression, any remodeling of the foxp3 gene by c-Rel must precede the formation of the Treg precursors. Many questions as to how c-Rel dictates Treg development remain to be answered, with the emerging data offering exciting insight into a previously unknown developmental role for c-Rel in mammalian cells that may provide mechanistic clues as to how c-Rel regulates other normal as well as pathological processes.

C-Rel has multiple roles in mature T cell growth, proliferation, and survival. Naïve T cells exiting the thymus are maintained in the periphery through a combination of cytokine and weak TCR signals induced by self-antigens, the latter at levels below the threshold required to promote cell cycle entry. Typically, the activation of naïve T cells involves antigen-presenting cells (APCs) such as dendritic cells delivering dual antigen/MHC and B7 co-stimulatory signals via the TCR and CD28, respectively, with CD28 optimizing TCR signals to reduce the threshold for antigen-dependent T cell activation. Depending on the type of cytokines that activated T cells receive from an APC, naïve CD4 T cells can differentiate into Th1, Th2, Th17, and follicular helper T cells, each of which represents a different class of effector T cell.

Nuclear NF-κB activity in naïve T cells is minimal, and c-Rel is only present at low levels in the cytoplasm of resting T cells. With the homeostatic maintenance of naïve T cells unaffected by the absence of c-Rel, the preexisting c-Rel does not appear to serve a critical “housekeeping” function. Although TCR signals rapidly activate preexisting RelA in resting T cells, the cytoplasmic stores of c-Rel are not readily mobilized. This difference reflects the type of IκB proteins that bind to RelA versus c-Rel. RelA associates with IκBα, which is degraded in response to TCR signals, whereas c-Rel is bound to IκBβ, which is relatively resistant to degradation following TCR stimulation. Instead, TCR signals promote a delayed induction of c-Rel.
expression, resulting from NFAT-mediated activation of c-rel transcription. The different kinetics of c-Rel and RelA recruitment to the nucleus is indicative of a need to activate distinct NF-κB transcriptional targets in a temporally ordered manner during T cell activation. Why RelA, like NFAT, is in the first wave of transcription factors mobilized by TCR signals, whereas c-Rel is a secondary transcriptional effector, is still not known, and this temporal order contrasts with B cells, where c-Rel is among the first transcription factors activated by B cell antigen receptor (BCR) signaling.

IL-2, which is required for autocrine-dependent T cell proliferation, is induced by c-Rel in response to TCR and CD28 signaling. c-Rel controls il2 gene transcription by remodeling the chromatin structure of the locus to make it permissive for transcription, which is similar to the way that c-Rel is thought to control foxp3 transcription in Treg cells, suggesting that chromatin remodeling is a general feature of c-Rel-dependent transcriptional regulation. Although c-rel−/− T cells display a TCR plus CD28-dependent proliferative defect in culture due to a failure to express IL-2, the relatively normal IL-2-dependent T cell functions found in c-rel−/− mice in vivo suggest that IL-2 can be effectively regulated by c-Rel-independent pathways. Aside from its restricted role in controlling IL-2 transcription, c-Rel does not appear to serve a general, nonredundant function during T cell proliferation. Rather, the role of c-Rel in T cell expansion appears to be stimulus dependent. For example, c-Rel promotes the clonal expansion of Th1 effectors in a T cell–intrinsic manner during the immune response to Toxoplasma gondii but is dispensable for influenza-specific CD8 T cell division in infected mice. This differential need for c-Rel during T cell activation presumably reflects the type of stimulatory signals invoked by specific pathogens. Notwithstanding the different stimulus-specific requirements for c-Rel in T cell proliferation, T cells (like B cells) use c-Rel in an overlapping manner with another NF-κB transcription factor, in this case RelA, to induce the c-Myc-dependent growth of mitogen-activated T cells.

Although c-Myc rescues the c-rel−/−/rela−/− T cell growth defect, the failure of these cells to proliferate in response to TCR signals indicates that c-Rel and RelA play additional roles in the cell cycle that are independent of cell growth. TCR-activated CD4 and CD8 T cells display a differential dependence on c-Rel for survival. Whereas TCR activation of PKCθ employs c-Rel to promote the survival of CD8 T cells, the PKCθ/c-Rel pathway is dispensable for CD4 T cell survival. However, c-Rel in combination with NFκB1 is critical for the survival of activated CD4 T cells. This emphasizes how c-Rel is differentially employed by CD4 and CD8 T cells to provide TCR-induced survival functions.

c-Rel also controls the differentiation of CD4 Th cells during diverse immune responses that include central nervous system inflammation, islet allograft rejection, and microbial challenge by using both T cell autonomous and nonautonomous mechanisms. For example, Th1 cell development in experimental autoimmune encephalomyelitis involves c-Rel-induced expression of IL-12 by APC, yet in Toxoplasma gondii infection, this aspect of Th1 differentiation is dispensable, with c-Rel instead serving a T cell–intrinsic role. Follicular helper (FH) T cells are required for the formation and maintenance of germinal centers and play a key role in plasma cell and memory B cell differentiation. IL-21, a cytokine produced by activated CD4 T cells that is important for the expansion and differentiation of FH T cells, requires c-Rel for the transcription of the il21 gene. Consistent with this finding, c-rel−/− mice exhibit defective FH T cell development and germinal center formation.

Although IL-21 administration rescues the defect in development of c-rel−/− FH T cells, germinal center formation in c-rel−/− mice remains impaired, indicating an IL-21-independent role for c-Rel in the formation of these crucial secondary lymphoid structures.

Finally, c-Rel is important in the regulation of cytokine expression by activated T cells. In addition to promoting the T cell–specific expression of IL-2, GM-CSF, IL-3, IFN-γ, and IL-21 (Table 2), in naive CD4 T cells exposed to inflammatory cytokines, an exchange of the IκB factor that tethers c-Rel in the cytoplasm from IκBβ to IκBα creates a T cell that expresses cytokines at a faster rate and in larger amounts during subsequent TCR activation. This c-Rel priming mechanism that permits a T cell to respond rapidly and efficiently in an antigen-specific manner may serve as a temporary stop gap role during an immune response while a more robust T effector response has time to evolve.

**c-Rel plays a role in activated B cell growth, proliferation, and survival.** In keeping with c-Rel being dispensable for the antigen-independent B cell development that occurs in the bone marrow, c-Rel levels are low in B cell precursors, and p50/RelA is the major NF-κB dimer found in these cells. However, during the developmental transition from a pre–B cell to a naive mature B lymphocyte, an up-regulation of c-Rel and p50 expression causes p50/c-Rel to become the predominant NF-κB dimer in peripheral IgM-positive B lymphocytes. This shift to c-Rel-dominated NF-κB heterodimers in mature B cells highlights the importance of c-Rel in the events linked to the immune activation and differentiation of mature peripheral B cells.

B cell activation in response to antigens, Toll-like receptor (TLR) ligands, or T cell (CD40 ligand) signals typically promotes cell cycle entry and cell division, which are accompanied by isotype switching and antibody secretion. Entry of a mature B lymphocyte into the cell cycle requires a quiescent cell to initiate and coordinate multiple biochemical processes. If a B cell is not primed to enter the cell
cycle when it receives an activation signal, then programmed cell death appears to be initiated as a default option to eliminate any inappropriately stimulated cells. Indeed, upwards of 30% of normal B cells undergo apoptosis following BCR engagement, suggesting that, at any given time, a significant proportion of quiescent B cells are not correctly poised to respond to mitogenic signals. To counteract this situation, it has been proposed that activated B cells engage a survival pathway that provides a window of protection from apoptosis to those cells that are able to be biochemically or metabolically reconfigured to successfully enter the cell cycle.

In mature B cells, c-Rel serves a dual role of promoting cell division and survival when these cells are activated. Although BCR, CD40, TLR4, and TLR9 signals all rapidly mobilize c-Rel dimers in resting B cells, studies with c-rel−/− mice have established that the requirement of c-Rel for mitogen-induced B cell division and survival is stimulus dependent. Whereas c-Rel is largely dispensable for promoting these functions in TLR9-stimulated B lymphocytes (S. Gerondakis, unpublished results), c-Rel is essential for these roles in antigen receptor–activated cells. c-Rel has two distinct cell cycle functions in B cells. In BCR-activated cells, c-Rel promotes the transition from G1- to S-phase. The genes that c-Rel directly regulates to control this phase of the cycle remain to be determined, although c-Rel induction of transcription factor E2F3a and cyclin E have been proposed to play a role in G1-to-S phase progression. c-Rel also promotes cell growth during G1 by upregulating c-myc transcription, a step associated with ribosome biosynthesis that is essential for subsequent cell division. Once BCR-activated B cells enter S-phase, c-Rel appears to be dispensable for progression through the remainder of the cell cycle. Although the combined roles of c-Rel and p50 in B cell growth appear to be a common requirement for all mitogenic signals, the need for c-Rel to promote S-phase entry is more selective, with its importance in the hierarchy of mitogenic stimuli headed by BCR signaling.

In the absence of c-Rel, mitogen-activated B cells display elevated levels of apoptosis. c-Rel promotes the survival of these cells by inhibiting a cell intrinsic death pathway through the direct transcriptional induction of genes encoding the Bcl-2 family pro-survival proteins A1/Blf-1, and Bcl-xL. With Bcl-2 transgene expression able to block the apoptosis of activated c-rel−/− B cells, coexpression of the closely related pro-survival proteins A1 and Bcl-XL in activated B cells could represent a fail-safe mechanism. However, enforced expression of A1 alone only confers partial protection to BCR-activated c-rel−/− cells, indicating that A1 and Bcl-xL serve distinct survival roles. That A1 and Bcl-xL have distinct roles in BCR-activated cell survival is supported by their kinetics of induction in these cells. A1 expression is rapidly induced with kinetics that coincide with the initial nuclear induction of c-Rel, whereas Bcl-xL expression is delayed, instead tracking with the second wave of nuclear c-Rel expression observed in mitogen-activated B cells. Different survival roles for A1 and Bcl-xL in B cell activation could involve A1 protecting cells from apoptosis during cell cycle entry (G0-to-G1 transition), whereas Bcl-xL may protect B cells later in the cell cycle or during DNA rearrangement events linked to isotype switching.

Mitogenic activation, in conjunction with cytokine signals such as IL-4 or IFN-γ, promotes isotype switching in a process whereby the assembled V gene is expressed in conjunction with Cμ and different C H regions. This process uses nonhomologous DNA rearrangement between regions of repetitive sequence (switch or S-regions) that flank Cμ and downstream C H genes. TLR4 and cytokine signals specifically target different C H genes for rearrangement by using transcription to allow the switch recombination machinery access to the S-regions upstream of targeted C H loci. A 3′IgH enhancer located downstream of the C H locus also controls isotype switching, albeit via mechanisms that remain to be determined. c-Rel is necessary for efficient switching to IgG1 and IgE, with c-Rel promoting S-region transcription prior to DNA rearrangement. c-Rel interaction with the 3′IgH enhancer may also contribute to the efficiency of the switching process. Although DNA rearrangement during switching depends on B cell proliferation, a general role for c-Rel in B cell division is difficult to reconcile with the differential impact that the loss of c-Rel has on switching to specific isotypes. However, a genome-wide scan has shown that c-Rel is enriched on DNA at sites of recombination.

Role of c-Rel in Human Disease

REL as a susceptibility locus in human immune diseases. Multiple reports have recently linked SNPs (single-nucleotide polymorphisms) within or near the REL locus with an increased susceptibility to certain human autoimmune diseases and cancer. These REL-associated diseases include rheumatoid arthritis, celiac disease, psoriasis, ulcerative colitis, primary sclerosing cholangitis, and B cell lymphoma (Table 4).

The 2 REL polymorphisms associated with increased susceptibility to autoimmune disease are located within REL introns: one within intron 4 has been linked to rheumatoid arthritis and Crohn’s disease, whereas the SNP in intron 2 is associated with celiac disease. If these SNPs are relevant to these human diseases, their presence within REL introns implies that altered REL transcription or mRNA maturation increases susceptibility to autoimmune disorders. In one study of potential celiac disease patients, the REL polymorphism did correlate with increased REL protein expression. However, the ability to find a change in
susceptibility. Likely to become increasingly important in human disease indicates that polymorphisms in the NF-κB pathway are.

In vitro transformation of lymphoid cells. Based on the ability of the viral oncoprotein v-Rel to transform a variety of avian hematopoietic cells in culture, it is not surprising that chicken, mouse, and human c-Rel also have this transforming activity in primary chicken spleen cell cultures. Although full-length versions of chicken and human c-Rel can transform chicken lymphoid cells in vitro, their transforming activity is enhanced when either of the 2 C-terminal transactivation domains is removed. In contrast, removal of the entire transactivation domain of c-Rel or mutations that abolish DNA binding inactivates c-Rel’s transforming activity. Taken together, such studies indicate that c-Rel must bind to DNA and activate target genes to effect transformation and that chronic low-level induction of target gene expression—such as occurs with certain transactivation domain deletions—is optimal for transformation.

Role of c-Rel in Lymphoid Cell Cancer

REL expression that is associated with these SNPs is complicated by not knowing the cell type or the stage in disease development (initiation or maintenance) for which a change in REL expression is important. For example, although c-Rel has been shown to be required in mouse models of arthritis, the relative contribution of c-Rel expression in immune infiltrates (T cells, neutrophils, and eosinophils) versus stromal cells is unclear. Even though c-Rel expression is perceived as being strictly linked with hematopoietic cells, it is also normally expressed at low levels in endothelial and epithelial cells. In the case of skin epithelium, the loss of NF-κB activity of which c-Rel is one component leads to the development of potent inflammatory skin lesions, highlighting the possibility that a loss or reduction of REL contributes to oncogenesis in human B cells. At least for DLBCL, RELΔTAD1 changes the GCB-like marker CD10. These results provide strong support for REL contributing to oncogenesis in human B cells.

Table 4. Association of REL Alterations with Human Disease

<table>
<thead>
<tr>
<th>Disease</th>
<th>Changes</th>
</tr>
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<tbody>
<tr>
<td>Celiac disease</td>
<td>SNP</td>
</tr>
<tr>
<td>Inflammatory bowel disease</td>
<td>SNP</td>
</tr>
<tr>
<td>Psoriasis</td>
<td>SNP</td>
</tr>
<tr>
<td>Rheumatoid arthritis</td>
<td>SNP</td>
</tr>
<tr>
<td>Sclerosing cholangitis</td>
<td>SNP</td>
</tr>
<tr>
<td>Leukemia/lymphoma</td>
<td>Gene amplification, chromosomal rearrangement, mutation, SNP</td>
</tr>
<tr>
<td>Oral carcinoma</td>
<td>Gene deletion, gene amplification</td>
</tr>
<tr>
<td>Metastatic lung cancer</td>
<td>Gene amplification</td>
</tr>
</tbody>
</table>

*SNP = single-nucleotide polymorphism associated with the disease.

In contrast to c-Rel, overexpression of p50, p52, RelB, RelA, and a constitutively active version of IKKβ is not transforming in avian lymphoid cells. However, substitution of the RelA transactivation domain with that of v-Rel can confer transforming activity onto the RelA-v-Rel hybrid, even though the reciprocal hybrid protein (v-Rel-RelA) cannot transform chicken lymphoid cells. This result suggests that the unique ability of c-Rel to transform avian lymphoid cells in culture resides in its C-terminal transactivation domain. Nevertheless, there is clearly flexibility in the type of transactivation domain that can induce avian lymphoid cell transformation when fused to an appropriate RHD, given that there is only about 10% sequence identity between the transactivation domains of chicken and human c-Rel and that the herpes virus activator protein VP16 can also substitute for the REL transactivation domain in transforming assays.

There have been no reports of v-Rel or c-Rel being able to transform mouse lymphoid cells in culture. Overexpression of a human REL mutant (RELΔTAD1) missing one transactivation domain can, however, enhance the oncogenic properties of the human B lymphoma cell line BJAB. Furthermore, RELΔTAD1 changes the GCB-like mRNA expression profile of BJAB cells into one more similar to the ABC-like DLBCLs, including increasing the expression of REL targets such as BCL2, IRF4, and miR-155, as well as reducing the expression of the GCB-like marker CD10. These results provide strong support for REL contributing to oncogenesis in human B cells.

REL gene amplifications in human B and T cell malignancies. The REL gene is located at human chromosomal position 2p16.1-15, which is a common site of gene amplification in a variety of B and T cell malignancies (Table 5). Among B cell lymphomas, amplifications of REL have been found at relatively high frequency in Hodgkin’s lymphoma (~46%) and non-Hodgkin’s B cell lymphomas, such as diffuse large B cell lymphoma (DLBCL) (~15%), Burkitt’s lymphoma (~7%), and follicular (~17%) and mediastinal (~21%) lymphoma. At least for DLBCL, REL appears to be the only gene in the minimally amplified region. In one follicular lymphoma, double minute chromosome amplification of
REL has also been reported. Overall, REL gene amplifications occur primarily in lymphomas with a mature B cell phenotype, which corresponds to the stage in development where c-Rel plays a major role in normal B cell function. Surprisingly, only one B lymphoma cell line has been reported to have REL gene amplification. REL gene amplifications have also been found in some leukemias (chronic lymphocytic leukemia) and T cell lymphomas (peripheral T cell, anaplastic large cell, natural killer).

Gene expression profiling studies indicate that increased NF-κB target gene expression and sensitivity to NF-κB pathway inhibitors can be used to classify one class of aggressive DLBCL—namely, the so-called activated B cell–like (ABC) DLBCL. Curiously, REL gene amplifications have been reported in one study to be confined to the germinal center B (GCB) subtype of DLBCL, which does not have particularly high NF-κB target gene expression and is generally resistant to NF-κB inhibitors. The restriction of REL gene amplifications to GCB-like DLBCL led Shaffer et al. to suggest that increased REL activity, as provided by REL gene amplification, is required at an early initiation stage of GCB DLBCL development but not for late-stage maintenance of tumor cell growth. However, independent analyses of DLBCL samples have found REL gene amplifications equally distributed among both the ABC and GCB molecular subtypes and have suggested that REL amplifications are more strongly associated with MYC gene rearrangements than with either the ABC or GCB subtype. Alternatively, REL gene amplification may be required for GCB-like DLBCL cell growth in vivo but not in vitro. Of note, there are no human DLBCL cell lines with high-level REL gene amplification, indicating that high-level REL gene/protein expression may be incompatible with in vitro DLBCL cell growth. Indeed, in some situations, high-level expression of REL can induce cell cycle arrest. Overall, the frequency of REL gene amplification in human lymphoma is striking; nevertheless, the role that REL gene amplification plays in DLBCL is still not entirely clear.

**REL gene rearrangements and point mutations in B cell lymphoma.** Experiments in chickens and mice established that the c-rel gene could be activated by retroviral insertional mutagenesis in B cell lymphoma. REL gene rearrangements and mutations have also been detected in human B lymphoma samples and cell lines. The RC-K8 DLBCL cell line has a large deletion on chromosome 2, which results in the expression of a chimeric REL protein (REL-NRG) in which the REL DNA-binding/dimerization domain is fused to sequences of unknown function (Non-Rel-Gene). Similarly, in one Hodgkin’s lymphoma sample, a genomic alteration near the 3' end of REL results in the expression of a C terminally truncated REL protein that is constitutively nuclear. In another Hodgkin’s lymphoma sample, REL is translocated to a position near the light chain enhancer, and in a third Hodgkin’s lymphoma cell line, an EBV genome has integrated near to REL, resulting in increased REL mRNA expression. Lastly, a point mutation that converts Ser-to-Pro at amino acid 525 within the transactivation domain of REL has been detected in 2 human B cell lymphomas. In one lymphoma patient, the S525P mutation is a germ-line mutation, suggesting that it is a predisposing mutation for lymphoma. The REL-S525P protein shows increased transforming ability in chicken spleen cells and altered transactivation properties. It would not be surprising if other human B cell lymphomas, with or without REL gene amplification, have activating mutations in REL, especially given that a variety of point mutations and deletions within the REL transactivation domain can enhance the in vitro transforming activity of REL.

Of particular interest, the RC-K8 DLBCL cell line has (at least) 4 mutational events that affect the REL/NF-κB pathway: (1) a deletion that results in the expression of the chimeric REL-NRG protein; (2) inactivating mutations in the gene encoding IκBα; (3) inactivating mutations in A20, an upstream negative regulator of NF-κB signaling; and (4) a disabling mutation in the REL transcriptional co-activator p300. As a consequence of these mutations, RC-K8 cells contain high levels of nuclear κB site DNA-binding complexes consisting of wild-type REL and NRG, and several REL target genes, including BCL-x, TRAF1, Bfl-1/A1, and ICAM-1, are expressed at high levels. Reexpression of IκBα blocks the proliferation of RC-K8 cells. On the basis of these findings, it has been proposed that constitutive REL-directed gene expression is required for the growth and survival of RC-K8 cells and that this chronic gene induction is tuned to an optimal oncogenic level by cooperation with nonactivating REL-NRG dimers and defective p300 protein.

**REL mRNA and protein expression in B cell lymphoma.** The findings described above suggest that the aberrant and chronic REL-induced gene expression seen in B cell lymphoma leads to enhanced mature B cell proliferation and survival. In some cases, this simple hypothesis is supported by experimental data. For example, many REL target genes are overexpressed in de novo DLBCLs and increased REL mRNA expression has been correlated with a poorer prognosis in splenic marginal B cell lymphoma. Moreover, down-regulation of REL expression by siRNA or chemical inhibitors has been shown to block B cell lymphoma growth. However, Kluiver et al. did not find a correlation between REL amplification and high-level REL mRNA expression in classical Hodgkin’s lymphoma.

REL protein expression or nuclear activity in primary human B cell lymphomas has been less thoroughly analyzed than REL mRNA expression. Curry et al. analyzed 68 newly diagnosed DLBCL samples and found that 65%
showed nuclear REL expression. Moreover, those patients with nuclear REL and the GCB-like gene expression profile had a worse overall survival. Houldsworth et al.93 did not find a correlation between nuclear REL expression and REL gene amplification—that is, some DLBCL samples with high-level REL gene amplification had less nuclear REL staining than some DLBCL samples with no REL gene amplification. Enhanced nuclear REL staining has, however, been reported to be a marker for certain types and stages of B cell lymphoma, as nuclear REL staining was reported in ~85% of Reed-Sternberg cells of classic Hodgkin’s lymphoma,115 in a majority of Hodgkin’s-like large cell lymphoma,116 and in mediastinal large B cell lymphoma.117,118 Furthermore, Rodig et al.119,120 presented data indicating that nuclear REL staining and high-level expression of the adapter protein TRAF1, a possible REL target gene product, can be used to distinguish classical Hodgkin’s lymphoma cells from other types of B cell lymphomas, such as anaplastic large cell lymphoma, lymphocyte-predominant Hodgkin’s lymphoma, and nonmediastinal DLBCL. Nevertheless, given that c-Rel complexes are normally in the nucleus of mature B cells, one cannot determine by immunolocalization whether nuclear REL staining in specific human B cell lymphomas is driving malignancy or is simply a marker for the developmental stage of the given tumor.

**Modulation of REL Activity for Therapeutic Purposes**

Given the role of REL in human cancer and autoimmune disease, modulation of REL activity or its transcriptional output could provide a therapeutic target. That inhibition of REL activity might prove a relevant strategy is bolstered by the facts that c-Rel expression is generally restricted to immune cells and that c-Rel knockout mice are fully viable (see above). Indeed, siRNA-mediated inhibition of c-Rel can block the growth of certain mouse B lymphoma cells,112 and knockdown of c-Rel can affect lung cancer cell growth in vitro.126 Moreover, c-Rel knockout mice show increased resistance to experimentally induced arthritis.75

In principle, specific inhibition of c-Rel could be achieved by down-regulation of c-Rel expression (e.g., by siRNA), by inhibition of a protein that is required for c-Rel expression or activity, or by inhibition of c-Rel transactivation activity (see Table 6). One compound with anti-inflammatory activity in clinical trials (STA-5326) has been reported to be a selective inhibitor of c-Rel nuclear translocation.131 Also, the immunosuppressant FK506 can

### Table 5. REL Gene Amplification in Human Lymphoma/Leukemia

<table>
<thead>
<tr>
<th>Lymphoma/Leukemia Type</th>
<th>% Amplified REL (Total Cases Analyzed)</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>B cell malignancies</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hodgkin’s</td>
<td>46 (175)</td>
<td>101, 188-192</td>
</tr>
<tr>
<td>DLBCL</td>
<td>15 (1379)</td>
<td>90, 91, 94, 118, 193-207</td>
</tr>
<tr>
<td>Follicular</td>
<td>17 (310)</td>
<td>99, 193, 197, 208-212</td>
</tr>
<tr>
<td>Primary mediastinal</td>
<td>21 (136)</td>
<td>91, 118, 199, 202, 213, 214</td>
</tr>
<tr>
<td>Cutaneous B cell</td>
<td>63 (31)</td>
<td>215</td>
</tr>
<tr>
<td>CLL</td>
<td>5 (525)</td>
<td>193, 211, 216-219</td>
</tr>
<tr>
<td>Lymphoplasmacyctic</td>
<td>10 (10)</td>
<td>211</td>
</tr>
<tr>
<td>Burkitt’s</td>
<td>7 (45)</td>
<td>193, 220, 221</td>
</tr>
<tr>
<td>Marginal zone</td>
<td>7 (42)</td>
<td>195, 211</td>
</tr>
<tr>
<td>Pythorax associated</td>
<td>28 (7)</td>
<td>198</td>
</tr>
<tr>
<td>T cell malignancies</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Peripheral T cell</td>
<td>11 (47)</td>
<td>222</td>
</tr>
<tr>
<td>Cutaneous CD30+ anaplastic large cell</td>
<td>75 (8)</td>
<td>223</td>
</tr>
<tr>
<td>Natural killer T cell</td>
<td>40 (5)</td>
<td>224</td>
</tr>
</tbody>
</table>

CLL = chronic lymphocytic leukemia; DLBCL = diffuse large B cell lymphoma.
which c-Rel plays a role in T cells, B cells, and other cell types. Normal biological processes (green boxes) and pathologies (red boxes) in amplification, will uncover mutations in REL gene lymphoid cell malignancies, with or without future genome-wide mutational screenings of other human malignancies and autoimmune diseases. It seems likely that downstream pathway are altered in many T and B cell gene and its B cell proliferation (Fig. 2). Moreover, the REL of regulatory T cells and in the regulation of mature T and system, especially in terms of its roles in the development c-Rel is an important component of the mammalian immune Concluding Remarks

c-Rel is an important component of the mammalian immune system, especially in terms of its roles in the development of regulatory T cells and in the regulation of mature T and B cell proliferation (Fig. 2). Moreover, the REL gene and its downstream pathway are altered in many T and B cell malignancies and autoimmune diseases. It seems likely that future genome-wide mutational screenings of other human lymphoid cell malignancies, with or without REL gene amplification, will uncover mutations in REL. Therefore, direct or downstream inhibitors of REL signaling may have therapeutic uses in human immune cell diseases.

Table 6. c-Rel Inhibitors

<table>
<thead>
<tr>
<th>Compound</th>
<th>c-Rel Activity Inhibited</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>STA-5326</td>
<td>Nuclear translocation</td>
<td>131</td>
</tr>
<tr>
<td>FK506</td>
<td>Nuclear translocation</td>
<td>132, 133</td>
</tr>
<tr>
<td>PIN inhibitor</td>
<td>Nuclear translocation</td>
<td>155</td>
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<tr>
<td>siRNA</td>
<td>c-Rel protein expression</td>
<td>112</td>
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<tr>
<td>Pentoxifylline</td>
<td>Anti-CD3-induced c-Rel expression</td>
<td>225</td>
</tr>
<tr>
<td>Epoxyquinoids</td>
<td>DNA binding</td>
<td>226</td>
</tr>
</tbody>
</table>

Figure 2. c-Rel in normal development and disease. Shown are the major normal biological processes (green boxes) and pathologies (red boxes) in which c-Rel plays a role in T cells, B cells, and other cell types.

specifically block c-Rel nuclear translocation in some cell systems. Direct inhibitors of c-Rel activity have not been identified. Given the similarity within the RHD between REL and other NF-κB family members, it is unlikely that sequences within the RHD could provide a specific therapeutic target; however, the REL transactivation domain, which is required for REL’s oncogenic and immune cell functions, might provide a specific target for inhibition. Finally, induction of REL activity could serve as a means of inducing Treg cell development to suppress autoimmune disease.

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Declaration of Conflicting Interests

The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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屯rel and cytoplasmic retention of p68

48. Harling-McNabb L, Deliyannis G, Jackson DC, Gerondakis S, Grigoriadis G, Brown LE. Mice lacking the transcription factor subunit Rel can clear an influenza infection and have functional anti-viral

82. Fan Y, Gélinas C. An optimal range of transcription potency is necessary for efficient cell transformation by c-Rel to ensure optimal nuclear localization and gene-specific activation. Oncogene. 2007;26:4033-8.


