SIGNALLING

A handle on the pre-BCR

Signal transduction by the pre-B-cell receptor (BCR) is a crucial checkpoint in B-cell development. However, the fleeting expression of the pre-BCR makes it difficult to investigate. Recently, a study in *Immunity* showed that PAX5-dependent activation of B-cell linker (BLNK) is a pivotal event in pre-BCR signalling. This discovery has led to the development of a new system to study the pre-BCR.

PAX5 is a transcription factor that is essential for B-cell development: without it, lymphoid progenitors cannot commit to the B-cell lineage and development is stalled at the pro-B-cell stage. This block seems to be partly due to a failure to rearrange and express the μ-chain of the pre-BCR. However, a rearranged μ-transgene does not rescue B-cell development in *Pax5*$^{-/-}$ mice. It turns out that there is no pre-BCR signalling in *Pax5*$^{-/-}$ μ-transgenic pro-B cells. So, PAX5 is probably also required for the expression of a crucial signalling component, which Schebesta and colleagues in Vienna set out to identify.

An established system in which PAX5 is fused to the oestrogen receptor (ER) was used to identify new PAX5 target genes in pro-B cells. *Pax5*$^{-/-}$ pro-B cells that expressed the PAX5–ER fusion protein were treated with oestrogen, which resulted in PAX5 activation. PAX5-induced transcripts were enriched by DNA subtraction, and several clones of *BLNK*, which encodes a B-cell adaptor molecule, were isolated. Two PAX5-binding sites were identified in the *BLNK* promoter and this gene was shown to be a direct target for PAX5.

Surprisingly, however, a *BLNK* transgene did not rescue the defect in B-cell development in *Pax5*$^{-/-}$ μ-transgenic mice. Although the reconstitution of BLNK restored pre-BCR signalling functions in pro-B cells — including Ca^{2+} flux, pre-BCR internalization, *c*-Kit downregulation and proliferation — these signals were not sufficient to overcome the block in pro-B to pre-B cell differentiation, indicating that an additional PAX5-driven event is required.

Because they cannot differentiate, BLNK-reconstituted pro-B cells from *Pax5*$^{-/-}$ μ-transgenic mice are locked in a perpetual pre-BCR signalling mode, which neatly circumvents the technical problems that are associated with transient pre-BCR expression. The authors created an inducible pre-BCR signalling system by fusing BLNK to the ER. The treatment of *Pax5*$^{-/-}$ μ-transgenic pro-B cells with a hormone ligand activates the BLNK–ER fusion protein, leading to pre-BCR signal transduction. Microarray analysis of gene expression in this system showed that BLNK-dependent pre-BCR signals control genes that are involved in proliferation, signalling, *V(D)J* recombination and growth factor responses.

The authors hope that their new pre-BCR signal induction system will open up a more detailed understanding of transcriptional reprogramming at the pre-BCR checkpoint.

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References and links

ORIGINAL RESEARCH PAPER Schebesta, M., Pfeffer, P. L. & Busslinger, M. Control of pre-BCR signaling by PAX5-dependent activation of the *BLNK* gene. *Immunity* **17**, 473–485 (2002)

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