B CELL DEVELOPMENT

Stepping up PAX5 expression

The commitment of lymphoid cells to the B cell lineage and maintenance of a B cell phenotype require the transcription factor PAX5, which is expressed by B cells at all stages of differentiation, but is repressed in plasma cells. However, until now little has been known about how the expression of PAX5 is regulated. Meinrad Busslinger and colleagues identify an enhancer region in the *Pax5* locus and show that B cell-specific PAX5 activity depends on the stepwise activation of enhancer and promoter elements.

The authors generated transgenic mice expressing large bacterial artificial chromosomes (BACs) containing a green fluorescent protein (*Gfp*) reporter gene inserted into sequences of *Pax5*. Mice transgenic for a *Gfp* reporter BAC comprising –18 kb to intron 6 of *Pax5* (see the figure) expressed GFP throughout B cell development but not in plasma cells or non-B cells. When the sequence upstream of the *Pax5* promoter was deleted, the GFP expression pattern was retained, showing that any B cell-specific regulatory elements must lie within *Pax5*. In support of this, deletion of intron 5 resulted in minimal GFP expression by mature B cells.

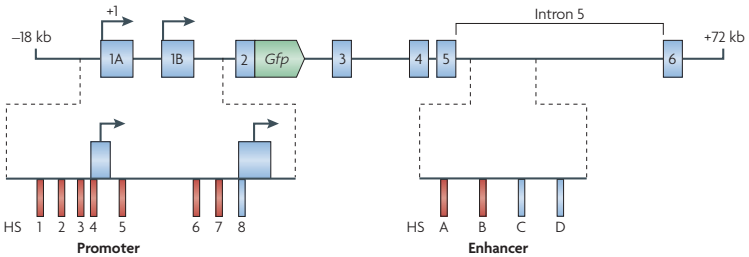
To localize potential regulatory elements in intron 5 of *Pax5*, the authors identified four DNase I hypersensitivity sites (HS) in this region (which indicate areas of active transcription). Two of these sites (HS-A and HS-B) were specific to B cells (shown in red). A region of *Pax5* from intron 4 to intron 5 containing HS-A and HS-B sites was associated with active chromatin modifications on histone H3 in pro-B cells that coincide with the known histone modification pattern of an active enhancer. A transgene containing the entire *Pax5* promoter region fused to a *Gfp* reporter gene was only expressed by mature B cells when HS-A and HS-B were inserted downstream of the *Gfp* gene. So, the regulated expression of PAX5 by B cells requires both the promoter region and the newly discovered enhancer region in intron 5.

Seven B cell-specific HS sites were also identified in the *Pax5* promoter (shown in red). As the transcriptional regulators E2A (encoded by *Tcfe2a*) and EBF1 are thought to act upstream of *Pax5* during B cell differentiation, the authors investigated the role of these factors in activating the *Pax5* locus. All seven HS sites were absent from the *Pax5* promoter in *Tcfe2a*$^{-/-}$ and *Ebf1*$^{-/-}$ B cell progenitors, and the chromatin at these sites had an inactive histone modification pattern. As E2A is expressed by *Ebf1*$^{-/-}$ progenitors (but EBF1 is not expressed by *Tcfe2a*$^{-/-}$ progenitors), it seems that EBF1 (and not E2A) is required for the formation of HS sites and activation of chromatin in the *Pax5* promoter. EBF1 was shown to regulate the *Pax5* promoter by binding with high affinity to HS-7.

By contrast, all HS sites in the *Pax5* enhancer were still present in *Tcfe2a*$^{-/-}$ and *Ebf1*$^{-/-}$ B cell progenitors and had active histone modifications, indicating that the enhancer region is normally active and accessible before EBF1 activity. HS-B of the *Pax5* enhancer provided binding sites for PU.1, interferon regulatory factor 4 (IRF4) and IRF8 in pro-B cells and mature B cells, and also binding of nuclear factor-κB in mature B cells.

In summary, the authors propose a model of B cell commitment whereby EBF1 expression at the onset of B cell development regulates chromatin remodelling of the *Pax5* promoter, which facilitates interaction of the promoter with the pre-activated enhancer region.

Kirsty Minton



Pax5–Gfp BAC. Image coutesy of M. Busslinger, Research Institute of Molecular Pathology, Vienna Biocenter, Austria.

ORIGINAL RESEARCH PAPER Decker, T. *et al.* Stepwise activation of enhancer and promoter regions of the B cell commmitment gene *Pax5* in early lymphopoiesis. *Immunity* 2 Apr 2009 (doi:10.1016/j.immuni.2009.01.012)