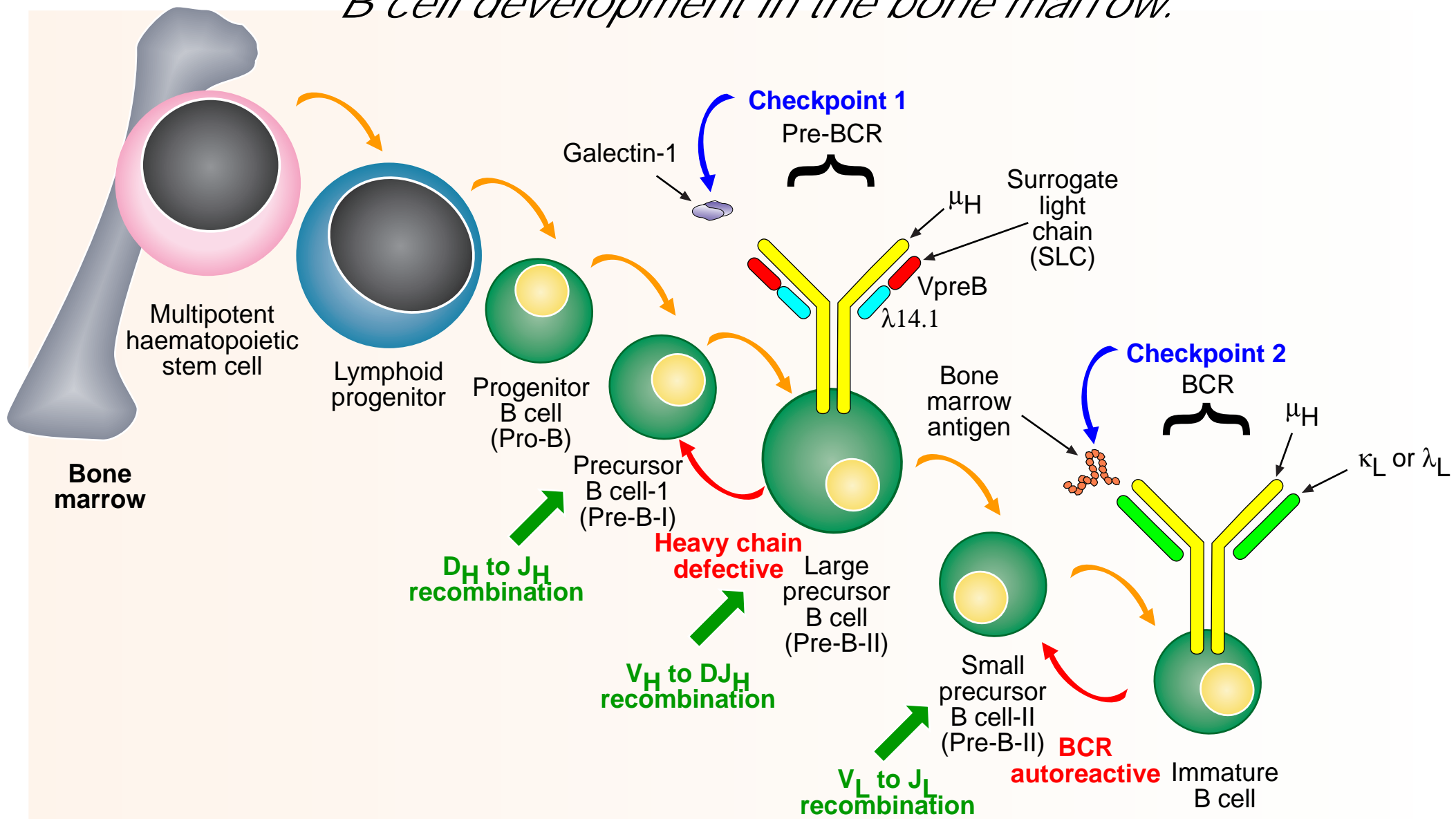
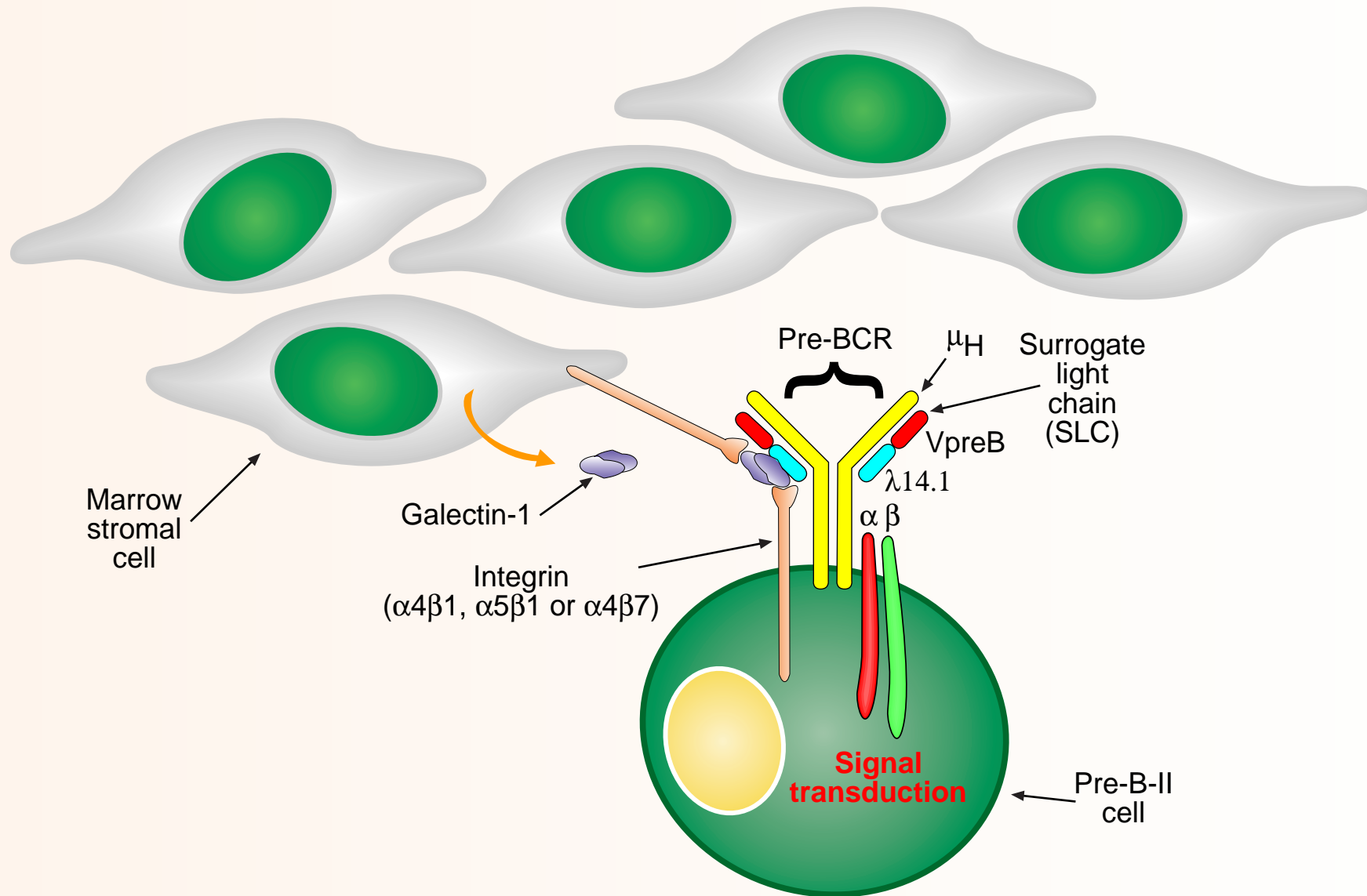


# *B cell development in the bone marrow.*



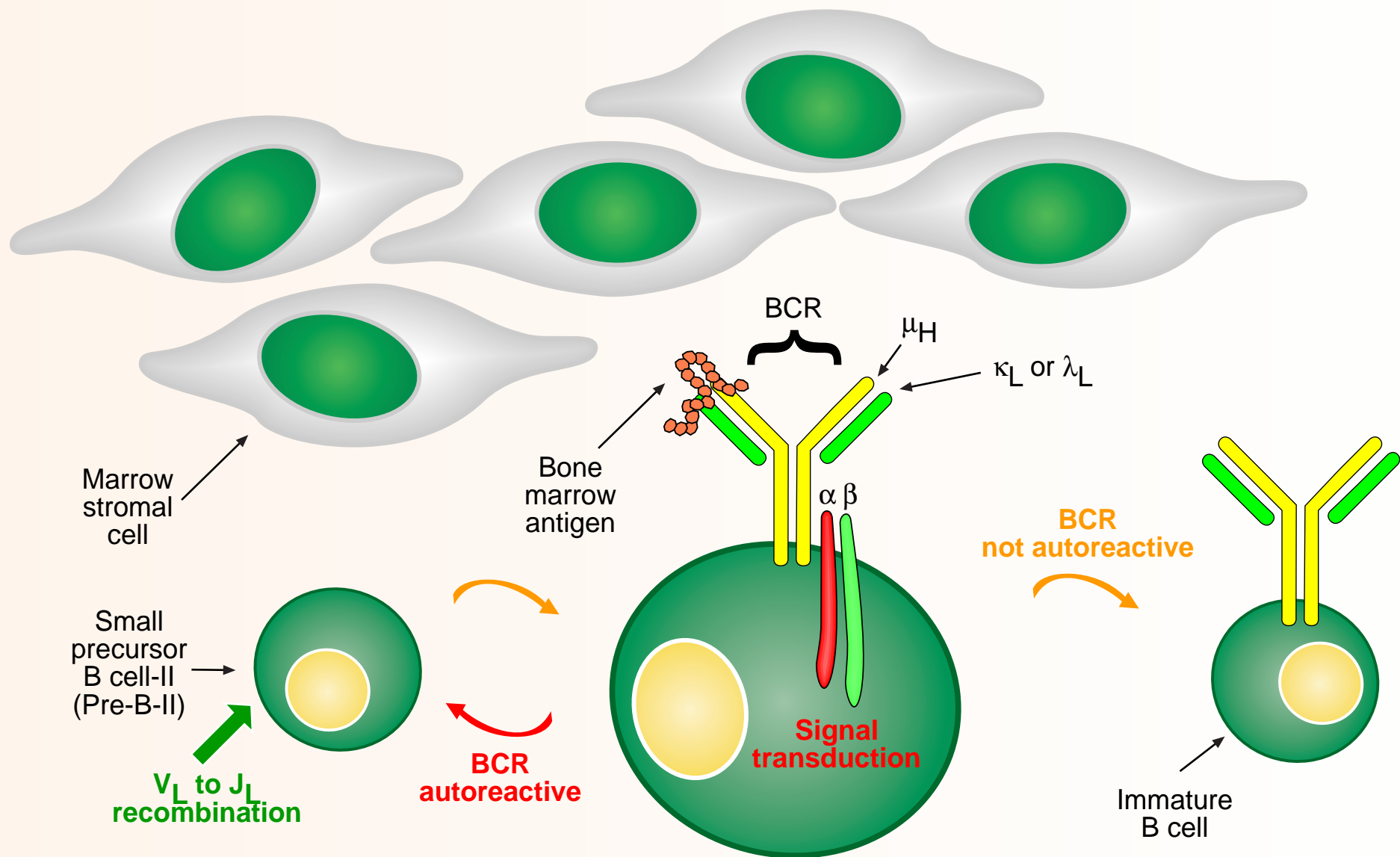
B cells develop from multipotent haematopoietic stem cells in the bone marrow that produce lymphoid progenitors which in turn generate B cell precursors. B cells with a unique antibody specificity develop by initial D to J rearrangement of the  $\mu$  heavy chain followed by V to DJ joining. The heavy chain pairs with the surrogate light chain (SLC) made of  $\lambda_{14.1}$  and VpreB to form the pre-BCR. Failure to assemble a functional pre-BCR prompts rearrangement of the second  $\mu$  heavy chain or the cell dies. A functional pre-BCR capable of signal transduction is tested with a stromal cell-derived ligand, galectin-1. Pre-BCR signaling failure prevents further B cell development (checkpoint 1). Next, rearrangement of the  $\kappa$  light chain occurs that pairs with the heavy chain to form the BCR. Autoreactivity is tested with bone marrow-derived antigens (checkpoint 2). An autoreactive BCR prompts rearrangement of the second  $\kappa$  light chain or else the  $\lambda$  light chains are used.

# Pre-BCR ligand binding and signal transduction (checkpoint 1).



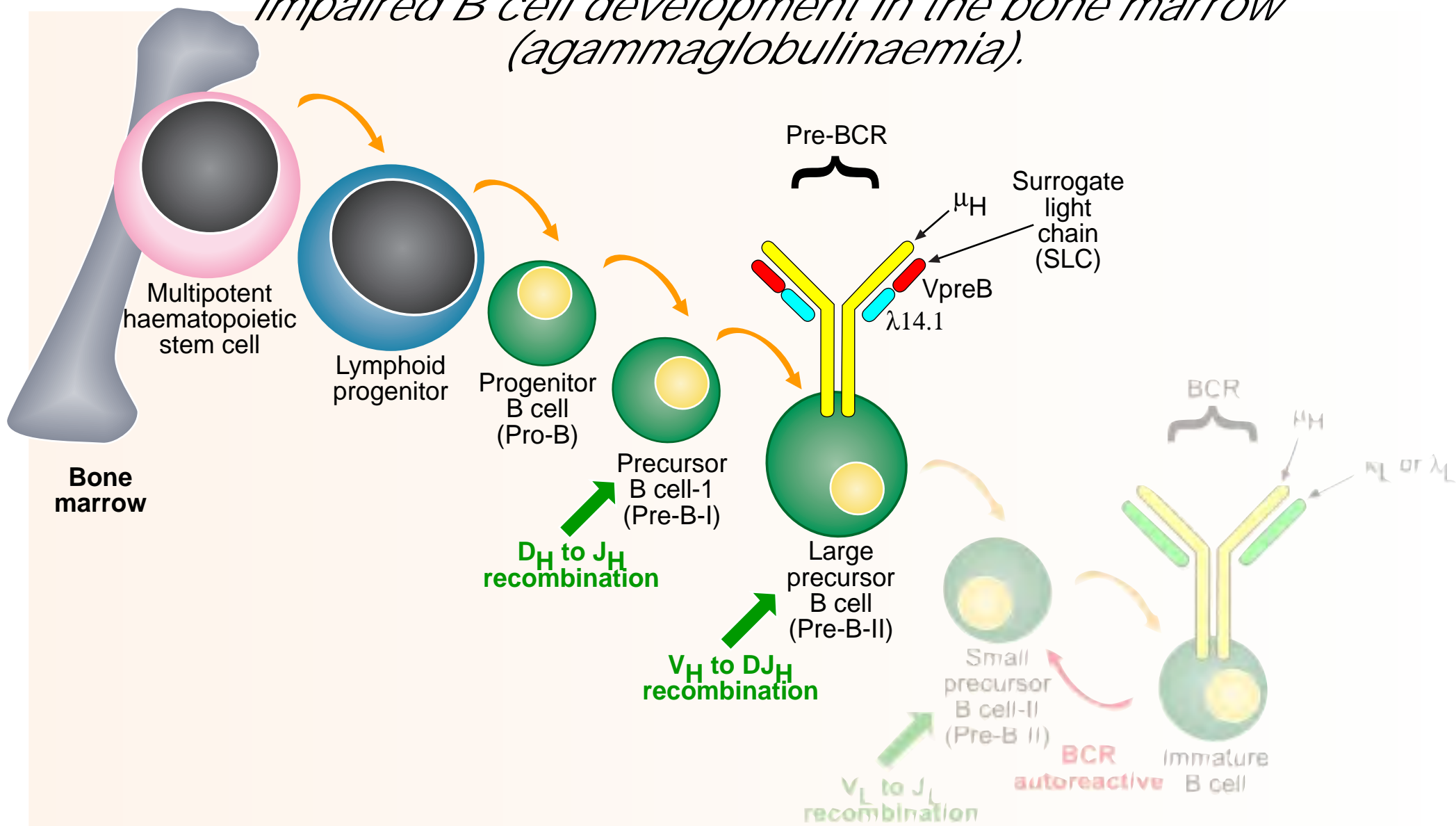
There are two checkpoints in the development of B cells to test the functionality of the BCR and to prevent the development of autoreactive B cells. The first checkpoint occurs after the rearrangement of a functional  $\mu$  heavy chain which pairs with the surrogate light chain (SLC) made up of proteins  $\lambda$ 14.1 and VpreB. This forms the pre-BCR which is expressed on the B cell surface where interaction with the protein ligand, galectin-1, secreted by bone marrow stromal cells, occurs. Galectin-1 is captured by cell-surface integrins (including  $\alpha$ 4 $\beta$ 1,  $\alpha$ 5 $\beta$ 1 or  $\alpha$ 4 $\beta$ 7) and also binds to the  $\lambda$ 14.1 protein of the SLC. Binding of galectin-1 to SLC initiates signal transduction via the Ig $\alpha$  and Ig $\beta$  transmembrane proteins that associate with the pre-BCR. Successful intracellular signaling events then prompt the B cell to rearrange and express a functional light chain. Failure to transduce the pre-BCR signal results in arrest of further development of the B cell.

# BCR autoantigen binding and signal transduction (checkpoint 2).

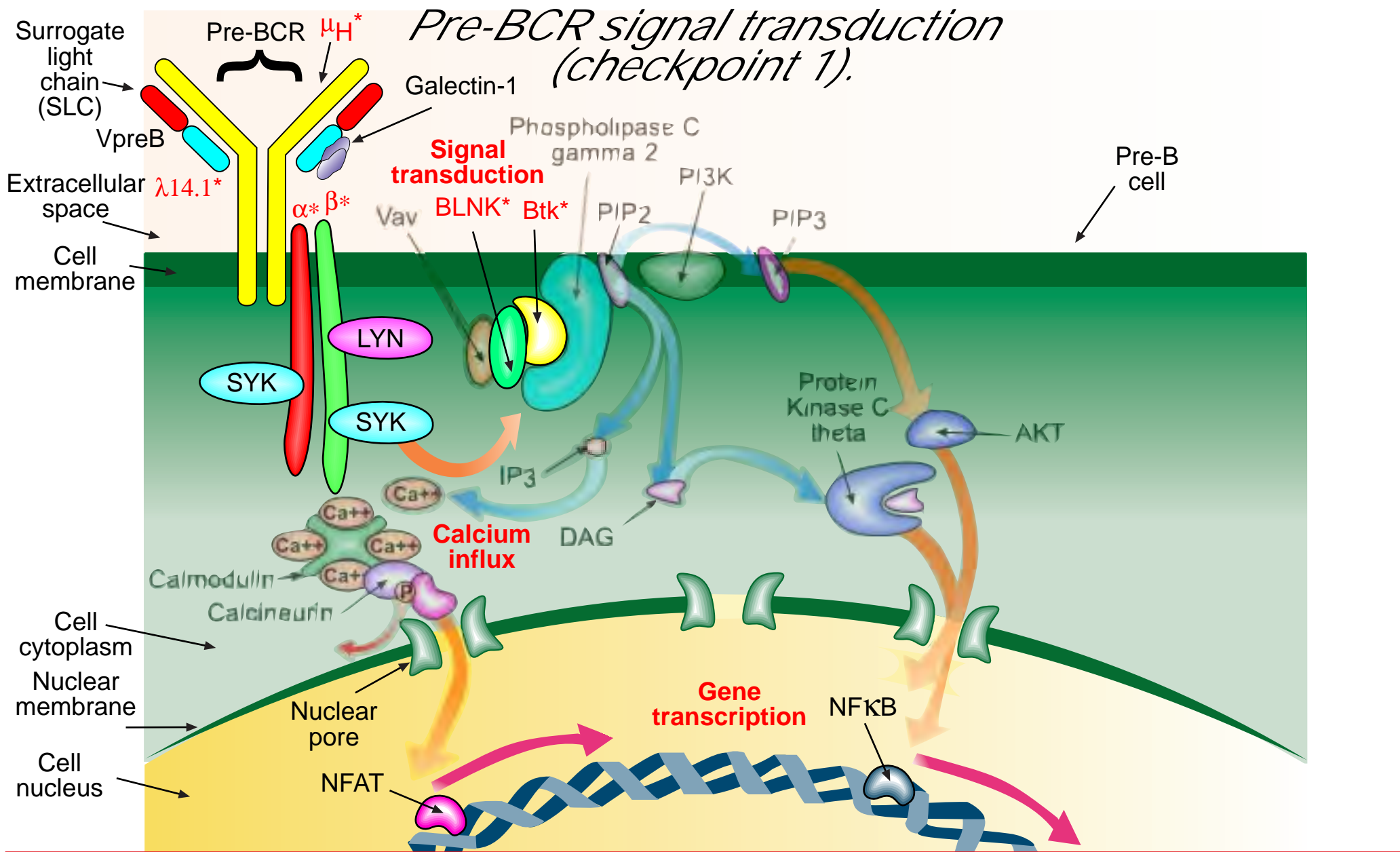


If signal transduction of the pre-BCR has been successful, the B cell initiates VJ recombination of the light chain that pairs with the heavy chain to form the BCR. The second checkpoint in B cell development is a test for potential autoreactivity. BCR's that recognise bone marrow-derived antigens with high affinity trigger an intracellular signal that prompts the B cell to rearrange an alternative light chain in order to generate a new BCR. The order of light chain rearrangements is usually the  $\kappa$  genes first followed by  $\lambda$ . Failure to generate a non-autoreactive BCR results in cell death. Successful B cells migrate out of the bone marrow to the secondary lymphoid organs where they may potentially detect foreign antigens.

# Impaired B cell development in the bone marrow (agammaglobulinaemia).

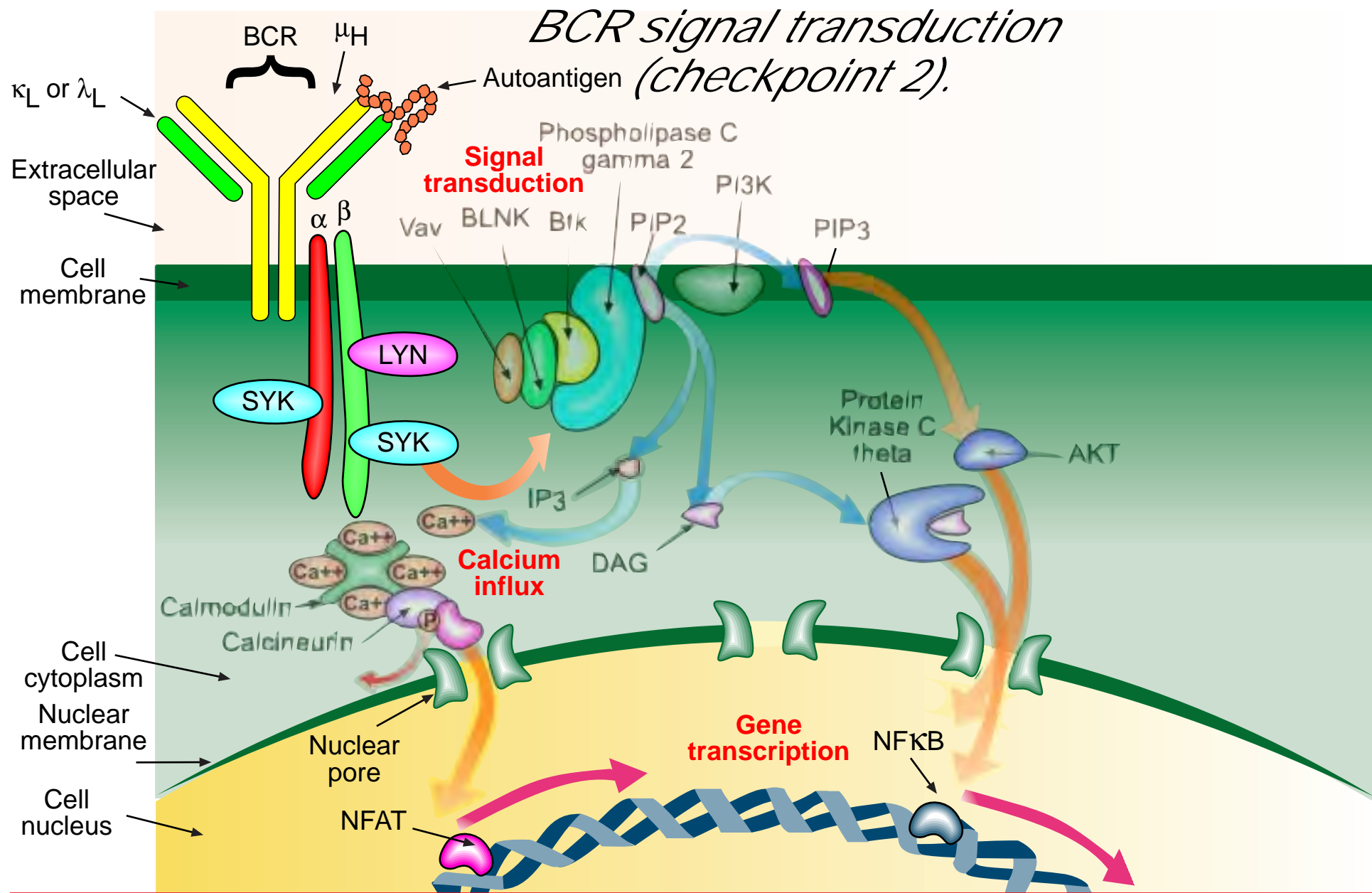


In primary immunodeficiency disease characterised by a lack of circulating antibodies as well as absent or low numbers of mature B cells there are often defects in the development of B cells in the bone marrow. A number of abnormalities have been identified in the intracellular signaling pathway at the first checkpoint during B cell development involving the pre-BCR. Failure to transduce signals from the pre-BCR following galectin-1 ligand binding prevents the precursor B cells from further development. This leads to reduced numbers or absent B cells in the periphery that produce IgM. There is also reduced opportunity for T-dependent activation of B cells which is a requirement for isotype switching to generate immunoglobulins such as IgG, IgA and IgE.



In greater detail, the intracellular signaling events following pre-BCR ligand-binding to galectin-1 is shown here with known defective proteins identified in agammaglobulinemia (indicated in red with an asterisk). The most common mutations (85%) cause Btk protein dysfunction. Btk is encoded by the X-chromosome and is referred to as sex or X-linked agammaglobulinemia. Pre-BCR signaling recruits SYK to the Igα and Igβ intracellular tails which in turn recruits BLNK. Mutations in BLNK are also known to cause agammaglobulinemia since this protein recruits Btk. Btk activates phospholipase C gamma-2 which is essential for the activation of transcription factors NFAT and NFκB required for gene transcription and further B cell development. Less common genetic mutations have been found in the μ heavy chain, λ14.1 and the Igα or Igβ transmembrane signal domains. Failure to transduce a signal from the pre-BCR results in arrest of further development of B cells in the bone marrow and the lack of B cells leads to the symptoms of agammaglobulinemia.

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The second checkpoint in B cell development also involves intracellular cell signaling, but this time the interaction of the BCR with bone marrow-derived antigens is indicative of autoreactivity. An autoreactive BCR prompts the B cell to rearrange an alternative light chain allele, usually in the order of the  $\kappa$  genes first followed by the  $\lambda$  genes. Failure to generate a non-autoreactive BCR will lead to cell death. Successful B cells will leave the bone marrow and migrate to secondary lymphoid tissues where foreign antigens can be detected. In agammaglobulinaemia this checkpoint may not be reached due to failure at checkpoint 1, however, some mutations are “leaky” and permit a reduced level of functionality of the affected proteins. This potentially affects checkpoint 2 where autoreactive B cells may escape elimination due to insufficient signaling of the BCR when contacting bone marrow self-antigens thus increasing the risk of developing antibody-mediated autoimmune reactions.