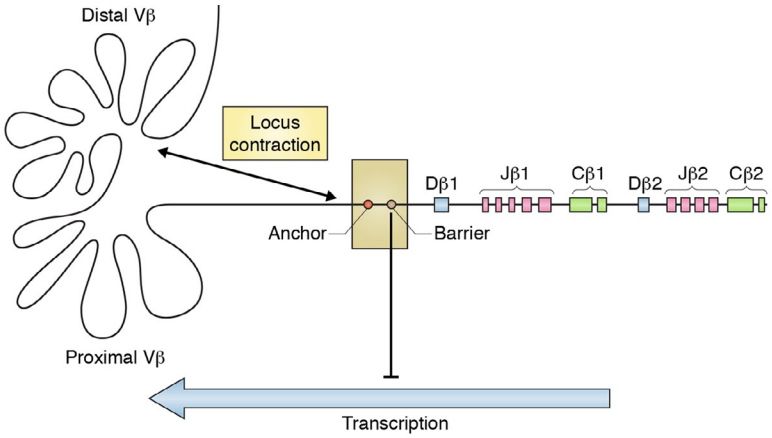
Ensuring an equal playing field for antigen receptor loci variable regions

Insight from Cornelis Murre

During the developmental progression of lymphoid progenitors, antigen receptor loci are assembled from variable (V), diversity (D), and joining (J) gene segments. In precursor B cells, the immunoglobulin heavy chain (*Igh*) locus undergoes ordered gene segment rearrangement with D_HJ_H joining preceding V_H to D_HJ_H rearrangement. Similarly, in progenitor T lineage cells, the T cell receptor β chain is assembled from V, D, and J elements, whereas the TCRα chain is generated from the joining of V and J elements. The ordered rearrangement of the *Igh* locus is controlled by an insulator element, characterized by two CTCF binding sites, named the intergenic control region 1 (IGCR1), located between the V_H and D_HJ_H region. The IGCR1 helps to equalize antibody repertoires by suppressing transcription of proximal VH regions and their recombination with D_H elements that have not yet joined with J_H regions. Likewise, the Vκ regions are segregated from the Jκ regions by regulatory elements, named Cer and Sis, which antagonize transcription across the proximal Vκ regions and suppress proximal VκJκ recombination.

In this issue, Majumder et al. provide new mechanistic insights into how regulatory elements help mediate appropriate long-range rearrangements between V elements and DJ regions involving the TCRβ locus. The authors dissect a genomic region separating the TCR Vβ from the DβJβ elements. They identify two elements within the insulator: a distally located high affinity CTCF biding site and a more proximal region characterized by relatively weak CTCF binding. They found that the most distally located CTCF-containing element functions as an anchor to promote looping of distal Vβ regions to the DβJβ region, essentially promoting locus contraction. The second element, located most proximally to the DβJβ region, acts as a barrier to prevent the spreading of active chromatin associated with the DβJβ region into the CTCF anchor. However, removal of the proximal element interferes with the ability of the distal element to promote locus contraction. Thus, these findings point to a separation of function for binding sites associated with insulator elements: anchors that promote long-range contraction must be protected from transcriptionally active chromatin by boundary elements.

The data presented by Majumder et al. indicate that the presence of a bifunctional insulator-anchoring element ensures an equal playing field for the Vβ repertoire. Such bifunctional elements are not restricted to the TCRβ locus. The IGCR1 element separating the V_H cluster from the D_HJ_H region and the Cer and Sis elements located in the *Igκ* locus may perform similar functions. Thus, a common mechanism is now emerging that underpins the generation of diverse antigen receptor repertoires for both B- and T lineage cells. Now, it will be important to unravel the mechanistic underpinnings of the bifunctional insulator-anchoring elements. For instance, how does the distally located anchor promote long-range contraction, and how does the proximally positioned boundary protect the anchor from active chromatin spreading in the DJ region? Further molecular analyses should reveal the mechanisms by which pairs of insulator-anchoring elements operate. It will be particularly interesting to examine whether and how trajectories adopted by the V and DJ regions across antigen receptor loci are controlled by the insulator-anchoring elements and how alterations in the paths taken by the chromatin fibers affect genomic encounters. Finally, the regulation of long-range genomic interactions—as described by this group and others—is not likely to be restricted to antigen receptor loci only. Rather, these studies will very likely serve as a paradigm for locus region encounters involving regulatory elements across genomes of both animal and plant kingdoms.



An anchor and a barrier separate the Vβ regions from the DJ clusters and ensure the generation of a diverse TCRβ repertoire.

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Re-tuning bone formation

Insight from
Gerard Karsenty

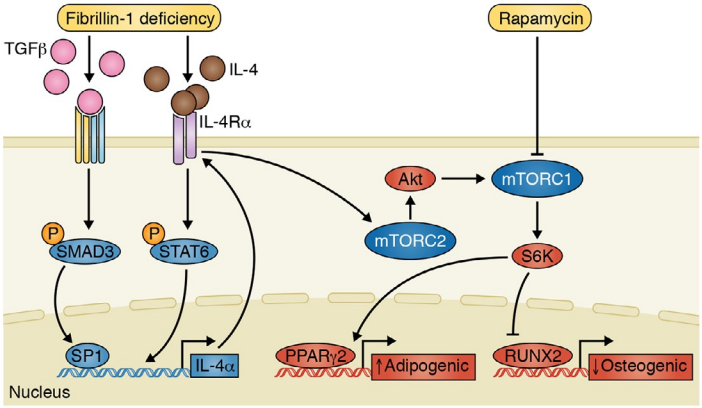
Fibrillin-1 (FBN1) is a structural component of microfibrils in the extracellular matrix that plays a role in tissue development. Genetic defects in *FBN1* are associated with a number of clinical conditions including systemic sclerosis (SSc)/scleroderma. In this issue, Chen et al. report that the osteopenia in a mutant "tight skin" mouse model of SSc (in which Fbn1 deficiency is due to a partial duplication of the *Fbn1* gene) is caused, at least in part, by an increase in IL-4 signaling in bone marrow mesenchymal stem cells (BMMSCs). This activation of IL-4 signaling in BMMSCs favors adipogenic differentiation and prevents osteoblast differentiation in an mTORC1-dependent manner.

Undoubtedly, this paper covers a lot of ground, from the bone pathology in this model of SSc, to the molecular pathogenesis and treatment with the mTOR inhibitor rapamycin. There is no doubt that the therapeutic aspect is the most original, exciting, and important one of this exhaustive study, mainly because it is the least expected.

First of all, this paper provides a long awaited explanation for the ability of IL-4 overexpression in cells of the osteoblast lineage to cause bone loss by decreasing bone formation, an observation that was first reported by the Perlmutter group 20 years ago. This is a very stimulating paper in a rapidly evolving field for several reasons. The model of Fbn1 mutation used in this paper is not a loss of function mutation. Hence, it will be important to determine if the increase in osteogenesis seen in Fbn1 loss of function mouse models will be explained by the same or by a different mechanism. Given the opposite nature of the bone phenotype in the two mouse models this is the most urgent question to address.

In a broader sense, the fact that activating the mTORC1 pathway would down-regulate osteogenesis is interesting because it seems counterintuitive. Indeed, osteogenesis is a process that requires a continuous and high level of protein synthesis; therefore, one would anticipate that activating the mTORC1 pathway would have a beneficial effect on osteogenesis, not a deleterious one. This is what has been observed in other, albeit different, mouse models, and it will be interesting to investigate the molecular basis of the differences between the tight skin mice and other models.

This study presents a large amount of data on the role of the FBN1/TGF-β/IL4Rα/mTOR cascade in BBMSC lineage selection. As acknowledged by the authors, it is clear that there is more to this story and that it is only the beginning of a long investigation. Further work to address the two key issues highlighted above will help to elucidate the regulation of bone formation by the extracellular matrix and the mechanisms whereby IL-4 signaling in cells of the osteoblast lineage can affect bone.



Fbn1 **induces alteration of BMMSC lineage commitment through the IL-4/mTOR cascade, leading to osteopenia in** ***Fbn1*** $^{+/-}$ **mice. Rapamycin restores BMMSC function and rescues osteopenia in** ***Fbn1*** $^{+/-}$ **mice.**

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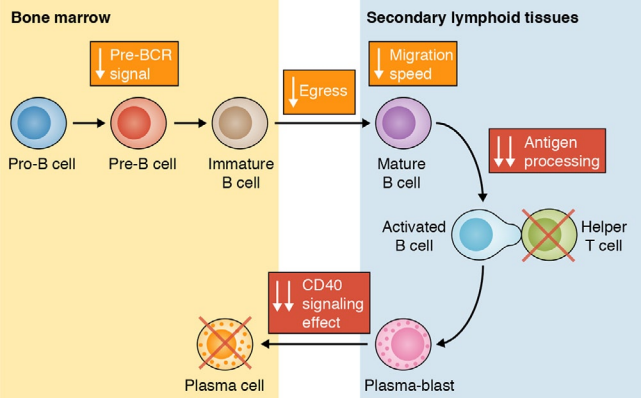
Pointing B cells in the right direction

Insight from
Michael Dustin

One of the most elegant master regulatory cascades in cell biology is the system of small G proteins controlling actin-based structures and coordinating these with cell polarity and cell cycle: Cdc42, Rac, and Rho. In this issue, Burbage et al. examine the role of one of these regulators, Cdc42, in B cell function using conditional gene targeting in mice. They find that this master regulator is everything it's cracked up to be—selective Cdc42 deletion in developing B cells led to a series of deficits that included decreased B cell receptor (BCR) signaling and delayed egress from the bone marrow, as well as an almost complete functional collapse when Cdc42-deficient B cells were asked to make antibodies in response to viral infection.



Roles of Cdc42 in B cell differentiation and function. Orange boxes represent a partial defect due to Cdc42 deficiency; red boxes indicate a profound defect in the absence of Cdc42.

Classical studies describe how stromal cells generate—in response to growth factors—fine, spiky protrusions called filopodia to probe the environment, ruffling protrusions to allow fluid uptake, and, finally, a network of stress fibers to exert contractile forces. The Rho family of small GTPases controls these distinct F-actin structures: Cdc42 for spikes, Rac for ruffles, and Rho for contraction. The generation of such structures is important for immune cells, as actin-based protrusions are the most sensitive part of cells for antigen recognition, and contractile force is an important ingredient in ligand recognition by T and B cell receptors.

In this study, Burbage et al. define roles for Cdc42 in development and mature B cell function. The first nonredundant role of Cdc42 is in pro-B to pre-B cell differentiation. Of potential interest, this is a transition for which CD19 is required, which might entail F-actin regulated recruitment of CD19 to pre-BCR signaling clusters. The authors also suggested that Cdc42 might be required for immature B cell responses to the egress signal, sphingosine 1 phosphate (S1P), although this was not directly investigated; such decisions could involve competition between retention and egress signals, either or both of which could contribute to the phenotype. However, an earlier study found that B cell chemotaxis to CXCL12 was intact in Cdc42-deficient B cells, which suggests that retention signals are intact. The most dramatic effects of Cdc42 deficiency uncovered in this study were found in mature B cells in response to antigen. Steady state levels of IgM and IgG were low in specific pathogen-free animals. Furthermore, mice with Cdc42-deficient B cells were unable to produce IgG antibody responses to flu infection. Vaccination revealed a 1 log defect in IgM production and 3 log defects in IgG isotypes, suggesting impaired T cell-dependent responses. BCR signaling, spreading and antigen uptake were impaired, but the partial defects could not obviously account for the defects in antibody production. Consistent with earlier reports, B cells appeared to localize normally in lymphoid tissues, but by using two-photon microscopy, the present study found that Cdc42 deficient B cells moved significantly slower in lymph nodes. Furthermore, the generation of MHC-peptide complexes was strongly impaired and there was a general failure of early T–B conjugate assembly and germinal center formation. It is known that plasmablasts display a strong, sustained polarization when exiting follicles for the medulla, where they begin maturation into plasma cells. While this aspect of plasmablast behavior was not studied, there was clearly a cell autonomous defect in the differentiation of plasma cells in response to CD40 engagement independent of the defect in T–B conjugation. Cdc42 was found to play a critical role in CD40L-induced activation of AP-1, which is required to up-regulate Blimp-1, a factor known to modulate plasma cell differentiation in B cells.

The mechanisms by which Cdc42 dependent signaling contribute to B cell development, loading of MHC-peptide complexes, and AP-1 induction in plasma cells are not known. It's also unclear how extensively quantitative defects in cell polarity are linked to these functional abnormalities. The defects in immature B cell egress and B cell migration may be secondary to polarity defects, but further studies are needed to determine if responses to chemotactic signals, such as S1P, are responsible and why this pathway would be more affected than G protein-coupled receptor–dependent responses to chemokines.

Burbage, M., et al., 2015. *J. Exp. Med.* http://dx.doi.org/10.1084/jem.20141143.

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