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NMP4, An Arbiter of Bone Cell Secretory Capacity And Regulator of Skeletal Response to PTH Therapy

Crystal Korff[1], **Emily Atkinson**[2], **Michele Adaway**[2], **Angela Klunk**[2], **Ronald C. Wek**[3], **Deepak Vashishth**[4], **Joseph M. Wallace**[5,6], **Emily K Anderson-Baucum**[7], **Carmella Evans-Molina**[7,8,9,10], **Alexander G. Robling**[2,6,9], **Joseph P Bidwell**[2,6]

[1]Department of Medical and Molecular Genetics, Indiana University School of Medicine (IUSM), Indianapolis, IN 46202

[2]Department of Anatomy, Cell Biology, & Physiology, IUSM

[3]Department of Biochemistry and Molecular Biology, IUSM

[4]Center for Biotechnology & Interdisciplinary Studies and Department of Biomedical Engineering, Rensselaer Polytechnic Institute, Troy, NY 12180

[5]Department of Biomedical Engineering, Indiana University-Purdue University at Indianapolis, IN, 46202

[6]Indiana Center for Musculoskeletal Health, IUSM

[7]Department of Pediatrics and the Herman B Wells Center for Pediatric Research, IUSM

[8]Center for Diabetes and Metabolic Disease and the Wells Center for Pediatric Research, IUSM

[9]Richard L. Roudebush VA Medical Center, Indianapolis, IN 46202

[10]Department of Medicine, IUSM

Abstract

The skeleton is a secretory organ, and the goal of some osteoporosis therapies is to maximize bone matrix output. *Nmp4* encodes a novel transcription factor that regulates bone cell secretion as part of its functional repertoire. Loss of *Nmp4* enhances bone response to osteoanabolic therapy, in part, by increasing the production and delivery of bone matrix. *Nmp4* shares traits with scaling factors, which are transcription factors that influence the expression of hundreds of genes to govern proteome allocation for establishing secretory cell infrastructure and capacity. *Nmp4* is expressed in all tissues and while global loss of this gene leads to no overt baseline phenotype, deletion of *Nmp4* has broad tissue effects in mice challenged with certain stressors. In addition to an enhanced response to osteoporosis therapies, *Nmp4*-deficient mice are less sensitive to high fat diet-induced weight gain and insulin resistance, exhibit a reduced disease severity in response to influenza A virus (IAV) infection, and resist the development of some forms of rheumatoid arthritis. In this review, we present the current understanding of the mechanisms underlying *Nmp4* regulation of the skeletal response to osteoanabolics, and we discuss how this unique gene

Corresponding author: Joseph P Bidwell, jbidwell@iupui.edu; Department of Anatomy, Cell Biology & Physiology, Indiana University School of Medicine, Indianapolis, IN 46202.

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contributes to the diverse phenotypes among different tissues and stresses. An emerging theme is that *Nmp4* is important for the infrastructure and capacity of secretory cells that are critical for health and disease.

Keywords

induced bone anabolism; osteoanabolics; osteoporosis; scaling factor; secretory cells; the unfolded protein response (UPR)

INTRODUCTION:

Nuclear Matrix Protein 4 (NMP4) was first identified as an osteoblast nuclear matrix protein that recognizes and binds to enhancers of the type I collagen gene[1,2]. It was also independently identified as Cas-interacting zinc finger protein (CIZ) and characterized as a nucleocytoplasmic shuttling factor able to associate with p130cas, a scaffold protein that mediates integrin and growth factor signaling[3,4]. The official name of this Cys_2His_2 zinc finger transcription factor is ZFP384 or ZNF384. In this review we will use the NMP4 (protein) and *Nmp4* (gene) designations unless specifically referring to clinical studies or investigations with human tissues/cells (ZNF384)[5].

NMP4 has the profile of a broadly important protein. It influences the expression of thousands of genes from a variety of families, binds to a large portion of the genome, and is highly conserved particularly between humans, primates, and rodents[6–8]. Nearly 300 organisms as disparate as the rufous-necked snow finch (*Pyrgilauda ruficollis*) and the Komodo dragon (*Varanus komodoensis*) harbor orthologues to *Nmp4*[5]. It is present in all tissues and appears to be constitutively expressed[6].

Remarkably, mice globally lacking the *Nmp4* gene are healthy and have no overt baseline phenotype[7–12]. However, they display enhanced bone formation in response to osteoporosis therapies[7–12], are less sensitive to high fat diet-induced weight gain and insulin resistance[13], exhibit a reduced disease severity in response to influenza A virus (IAV) infection[14], and resist the development of some types of rheumatoid arthritis[15]. *Nmp4* does not appear to be a redundant gene, i.e., the circumstance where two or more genes perform the same function or can partially or fully substitute for the mechanistic role of the other[16–20]. It is also not likely to be a dispensable gene, a gene that is not shared between all individuals of a species[21,22].

A potential role for *Nmp4* in linking these diverse preclinical scenarios may be to establish the capacity of secretory cells early in cell differentiation. The concept of the scaling factor was proposed as a mechanism by which the cell allocates a large portion of its proteome during early differentiation to meet the requirements of the nascent secretory cell[23–28]. These evolutionarily conserved transcription factors control large subsets of hundreds to thousands of genes and bias their expression towards the establishment of the cell's protein production and secretory machinery i.e., they program the capacity of the secretory cell in advance of the high demand for protein delivery[23,26,27,29]. Scaling factors can functionally cooperate with XBP1, a key transcriptional regulator of the unfolded protein response

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(UPR), to maximize the secretory capacity while allaying endoplasmic reticulum (ER) stress and apoptosis[27,29,30].

NMP4 has a similar functional profile to other scaling factors. Conditional loss of this gene in mesenchymal stem/progenitor cells (MSPCs)/osteoprogenitors, but not in later stages of osteoblast differentiation, supports the enhanced bone formation response to the osteoanabolic drug PTH[31]. NMP4 controls hundreds of genes involved in establishing and regulating the protein production and secretory machinery in bone cells including ribosome biogenesis, translation, and the UPR[7,8,32]. *Nmp4*$^{-/-}$ osteogenic cells exhibit elevated collagen secretion[7]. Additionally, *Nmp4*$^{-/-}$ MSPCs exhibit precocious mineralization in culture, a tightly controlled secretory process[7,8], whereas over-expression of *Nmp4* in an osteoblast cell line suppresses mineralization[33]. Although *Nmp4* is expressed in all tissues, its regulation of is tissue specific. Contrary to its role in osteogenesis, *Nmp4* supports pancreatic β-cell development and insulin secretion[13], the induction and release of chemokines from lung epithelial cells during IAV infection[14], and the secretion of IL-1 β, RANKL, and MMP-3 from joint cells during the development of arthritis[15].

In this review, we will primarily focus on the mechanisms by which *Nmp4* suppresses induced osteoanabolism. We will examine the evidence for the proposed scenario that NMP4 pre-programs the limits of bone response to osteoanabolic drugs by establishing protein production and secretory machinery early in osteoblast development. We will also evaluate the potential role of NMP4 in establishing the osteocyte secretory phenotype and non-bone secretory cells. Key gaps in our knowledge about NMP4 will be identified.

Severe osteoporosis is anything but “silent” and requires osteoanabolic therapy for bone restoration.

Key concept:

- Current FDA-approved drugs designed to restore bone lost to osteoporosis have limited clinical efficacy.

Osteoporosis is often referred to as the “silent disease” because the early pathogenesis typically goes unnoticed by affected individuals[34–36]. However, those afflicted with severe osteoporosis are often hobbled with pain and the complications of restricted mobility due to fractures[37,38]. This form of the disease is clinically defined as presenting with one or more fractures and a bone mineral density ≥ 2.5 SD below the young adult mean[39]. Severe osteoporosis is more prevalent in the old elderly (≥80 years of age)[40–43], and the population age 65 and over in the United States (US) is projected to almost double to 98 million by 2060[44]. Coincident with this greying of the US, the number of hip fractures, among the most devasting type of break, is expected to significantly increase over this same time period[45]. Finally, patients with severe osteoporosis are significantly more expensive to treat compared to those presenting with a milder form of the disease[46,47].

Osteoanabolics, drugs that add a significant amount of bone to the osteoporotic skeleton, are typically the initial therapy of choice for patients presenting with the severe form of the disease[48–50]. There are presently three FDA-approved osteoanabolics that exploit two clinical approaches: the first approach is the stimulation of the parathyroid hormone

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receptor with one of two parathyroid hormone (PTH) analogues (i) teriparatide [PTH 1-34] and (ii) abaloparatide or parathyroid hormone related peptide [PTHrp 1-34]. The second approach is achieved by the blockade of sclerostin, a natural inhibitor of Wnt signaling, with romosozumab-aqqg[51–53]. The efficacy of both strategies diminishes with time as all three drugs exhibit a 'treatment plateau', which limits bone gain[51–53].

The rapid loss of osteoanabolic therapeutic efficacy is problematic for treating a chronic degenerative disease. This obstacle has motivated research on enhancing the potency of these drugs to maximize their benefits. The first osteoanabolic approved by the FDA 20 years ago was PTH[54], and since this time several studies have been published describing efforts to boost its bone formation activity[55,56].

The principal strategy for increasing PTH anabolic action has been to enhance and/or extend the 'anabolic window' by the use of combination therapy, i.e. treating the patient with PTH followed by a drug that slows or stops bone resorption, such as an anti-resorptive/anti-catabolic drug[56]. Briefly, treatment with intermittent PTH or PTHrp, transiently elevates bone remodeling and enhances the bone formation arm over the resorption arm[52,57–62]. Initiation of treatment induces a surge of bone forming activity followed by a slower increase in resorption, the latter diminishing some of the initial skeletal gains. This phase is followed by a gradual drop of both processes to close to baseline levels. The anabolic window is the early phase of treatment in which formation significantly exceeds resorption[52,57–62]. Therefore, the rationale for combination treatment is that the use of an anti-resorptive agent along with or following PTH should mitigate the resorption arm's limiting action on the hormone's anabolic potency and preserve the newly acquired bone gains[56].

Exploring strategies for enhancing osteoanabolic potency led to the discovery of NMP4, a PTH-responsive, nuclear matrix MAR-binding/architectural transcription factor.

Key concept:

- NMP4 was discovered during a search for proteins that link PTH-induced changes in osteoblast collagen expression with the accompanying change in cell shape.

Early studies demonstrated that PTH induces changes in osteoblast morphology both *in vivo* and *in vitro*[63,64], which raised the question of whether such alterations in cell shape are instrumental in mediating the hormone-induced anabolic phenotype[65]. In the 1990s the concept of the tissue tensegrity-matrix was put forth and developed by several investigators[66–69]. Briefly, in this model the genome is literally "hard-wired" to the sub-structure of the adherent cell, i.e., there are physical links between the extracellular matrix, integrin receptors, the cytoskeleton, LINC proteins, and the nuclear matrix, which in turn, makes connections to the DNA[66–69]. These models gave us a novel conceptual framework from which to interrogate the molecular basis of PTH anabolic action (Figure 1).

A detailed examination of the nuclear matrix goes beyond the objectives of this review. Briefly, many of these proteins play roles in mechanotransduction and therefore may translate changes in cell/nuclear morphology and adhesion into changes in gene

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expression[70–73]. Nuclear matrix proteins also regulate gene expression via their role in mediating 3D genome organization[74,75]. For example, some of these proteins support anchor points to the DNA to form topologically confined chromatin domains. These matrix attachment regions (MARs) are often adenine/thymine-rich (AT-rich) and promote the assembly of multiprotein structures, which bend or loop the DNA thus mediating interactions between otherwise distant trans-activating factors and co-factors[76,77]. Similarly, some architectural transcription factors also bind to AT-rich sequences that are non-MAR sites and regulate gene expression by bending the local DNA[78,79]. We proposed extending the tissue tensegrity-matrix model to include nuclear matrix MAR-binding proteins or nuclear matrix architectural transcription factors, that convert changes in the shape of the cell into changes in the interactions of other trans-acting proteins at distal sites along target genes[80].

Therefore, since collagen expression is coupled to cell structure in connective tissue[81], we searched for and found a PTH-responsive, nuclear matrix, AT-rich binding protein that associates with the regulatory regions of the type 1 collagen gene, *Col1a1*[2]. Briefly, this binding protein, that we named NMP4, exhibited several characteristics of a MAR-binding protein and was later formally identified as such[2,82]. Additionally, exposure of bone cells to PTH increased NMP4 binding at these sites and exhibited DNA-bending activity[2]. These data provided a potential mechanism for converting PTH-induced changes in osteoblast shape into alterations in gene expression (Figure 2A).

Cloning and functional domain analysis of NMP4 revealed context-specific features and functions.

Key concept:

- NMP4 is a Cys_2His_2 zinc finger transcription factor and its AT-rich binding site may direct this protein to enhance, repress, or exert no regulatory effect on a particular target gene.
- This gene was independently cloned by two groups looking for novel genes involved in mechanotransduction.

Two groups independently cloned *Nmp4/CIZ* and we briefly describe both studies as it illustrates a common discovery during searches for novel genes involved in mechanotransduction[3,6]. We cloned *Nmp4* by isolating several full-length cDNAs from a UMR-106-01 expression library using one of the *Col1a1* enhancers as a probe[6]. These cDNAs encoded isoforms of a Cys_2His_2 zinc finger protein containing a well-defined DNA binding domain (5–8 Cys_2His_2 zinc fingers), two distinct transactivation domains located in the N- and C-termini, and an AT-hook domain[6,83,84] (Figure 2B). The NMP4 zinc finger domain serves multiple functions including binding to the AT-rich consensus sequence as well as acting as a nuclear localization signal and a nuclear matrix targeting signal[83,84]. The NMP4 homopolymeric (dA•dT) binding site exhibits modest sequence variability throughout the genome and its zinc fingers may recognize the local structural contour of the rigid narrow minor groove instead of the nucleotide sequences presented in the major groove, also known as ‘indirect readout’[85]. Trans-activation experiments with native and heterologous promoters revealed a sensitivity of the two transcriptional regulatory domains

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to their attached DNA-binding domain or to their DNA-binding state[83]. We concluded that the AT-rich consensus sequence can act as an allosteric ligand for NMP4 and provide the molecular basis for the observed context-specific/site-specific functionality[83], perhaps by altering its affinity for other ligands, e.g., coactivators or corepressors[86,87]. The NMP4 AT-hook domain, present in multiple architectural transcription factors and in some MAR-binding proteins, may also associate with the minor groove of the AT-rich consensus site and mediate the DNA bending[2,79].

The Hirai group was the first to clone this gene and found it in a search for ligands of p130cas (Cas), a focal adhesion protein proposed to transmit signals for the remodeling of actin stress fibers and cell movement[3]. Their data showed that CIZ shuttles between the nucleus and focal adhesions, thus providing another pathway for transmitting mechanical signals to target genes[3]. The extent to which CIZ/NMP4 localizes to focal adhesions and engages in nucleocytoplasmic shuttling is a key gap in our knowledge and requires further study.

Global loss of the gene *Nmp4* in experimental mice enhances the skeletal response to anabolic PTH therapy.

Key concept:

- Two global *Nmp4*$^{-/-}$ mice were independently engineered and both were observed to exhibit an enhanced response to osteoanabolics.

We engineered mice harboring an *Nmp4* global loss-of-function to test whether this gene regulates the skeletal response to anabolic doses of PTH[9]. These *Nmp4*$^{-/-}$ mice are healthy, and exhibit an unremarkable skeletal phenotype[7–12]. Global *Nmp4*$^{-/-}$ mice exhibited a strikingly enhanced PTH-induced increase in trabecular bone compared to their wild-type (WT) littermates[7–12]. The increase in PTH-induced trabecular bone formation did not occur at the expense of hormone-mediated increases in cortical bone[7–12]. Ovariectomized *Nmp4*$^{-/-}$ mice showed the same exaggerated skeletal response to anabolic doses of PTH[8,12]. These mice also had an enhanced bone formation response to the combination therapy of PTH + raloxifene[12]. Male mice were not tested for this response, but they do have an altered bone anabolic phenotype (see below).

Nakamoto et al., independently generated a global *Nmp4*$^{-/-}$ knockout mouse (Cas-interacting zinc finger protein, *CIZ*$^{-/-}$)[88]. These mice also showed an enhanced response to osteoanabolic challenge. Bone morphogenetic protein 2 (Bmp2) induction of bone formation on adult mouse calvariae *in vivo* was heightened in these mice compared to their wild type littermates[89]. They were also resistant to bone loss due to skeletal unloading[90]. Male mice, 10 weeks of age, were subjected to 2 weeks of tail suspension. Histomorphometric analysis showed that unloading suppressed bone formation in the wild-type mice but not in the *Nmp4 [CIZ]*-deficient mice[90]. Unloading-induced bone resorption was insensitive to *Nmp4* [*CIZ*] status[90].

We are currently investigating whether there is an enhanced anabolic response to skeletal loading in the absence of *Nmp4*. The rationale for these ongoing studies is that loading of bone by gravitational or muscle forces, can improve bone strength by stimulating

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adaptations in bone mass, shape and microarchitecture[91]. We have previously reported that NMP4 mediates changes in the expression of the extracellular matrix gene *Mmp-13* during the stimulation of MC3T3-E1 osteoblast-like cells to fluid shear stress, an early *in vitro* model of bone loading[92].

Bone marrow-derived *Nmp4*$^{-/-}$ mesenchymal stem progenitor cells (MSPCs) exhibit a cell autonomous precocious mineralization and elevated collagen secretion in culture.

Key concept:

- Loss of *Nmp4* biases MSPCs towards bone anabolism by the re-programming of large sets of genes controlling biosynthetic processes.

Analysis of the bone marrow cellular profile showed that the global *Nmp4*$^{-/-}$ mice exhibited a significant elevation in CFU-F cells, CFU-F$^{AlkPhos+}$ cells (osteoprogenitors), and a higher percentage of CFU-F$^{AlkPhos+}$ cells/CFU-F cells, consistent with an expanded population of MSPCs and osteoprogenitors[11]. Isolated bone marrow *Nmp4*$^{-/-}$ MSPCs from these mice showed a significant acceleration of mineralization in culture and increased type I collagen mRNA and protein expression compared to the same cells isolated from the WT littermates[7,8].

We probed the molecular pathways targeted by *Nmp4* in MSPCs, osteoprogenitors, and osteoblasts. Our genome-wide studies using ChIPseq and bulk RNAseq have provided insight into the *Nmp4* regulatory landscape[7,8]. The protein NMP4 binds to a considerable portion of the genome[8]. ChIPseq analysis of NMP4 DNA-binding in MC3T3-E1 osteoblast-like cells revealed that PTH treatment reduced NMP4 genome-wide occupancy from a total of 15,446 to 13,109 binding sites[8,93]. However, at the level of the single gene there was a diversity of changes in NMP4 occupancy, i.e., PTH was observed to remove, induce, or have no effect on NMP4-DNA association[8]. Consistent with our NMP4 functional domain analysis[83], the ChIPseq data suggest that NMP4 transcriptional responses to PTH are highly context specific. Gene ontology identified NMP4 target genes as enriched for negative regulators of biosynthetic processes, in agreement with the induced hyper-anabolic phenotype observed in both *Nmp4*$^{-/-}$ bone cells and *Nmp4*$^{-/-}$ skeleton[8].

The transcriptomes of *Nmp4*$^{-/-}$ MSPCs and osteoblasts supports bone cells with a high capacity for collagen secretion.

Key concept:

- *Nmp4* influences the expression of thousands of genes many of which regulate protein production, secretion, and the unfolded protein response (UPR).

Consistent with our ChIPseq data[8], we determined that *Nmp4* has a broad impact on the transcriptome of osteogenic cells[7]. *Nmp4*$^{-/-}$ MSPCs cultured in non-differentiating medium for 3 days displayed a significantly greater than or equal to two-fold change in the expression of 5032 genes compared to the *Nmp4*$^{+/+}$ cells[7]. Specifically, loss of *Nmp4* increased the expression of 3468 genes and decreased the expression of 1564 genes[7]. RNAseq analysis was also undertaken on MSPCs maintained in differentiating medium and harvested after 7 days in culture, coinciding with mineralization initiation[7]. At this time

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point in culture, the expression of 5313 genes were altered with 3925 exhibiting a significant increase and 1388 showing a decrease. *Nmp4* status had no influence on the expression of 8151 genes[7].

Ingenuity Pathway Analysis of our bulk RNAseq analysis predicted that loss of *Nmp4* elevates protein synthesis by enhancing the mRNA expression of several genes in the pathways of ribosome biogenesis, tRNA charging, amino acid synthesis, and translation initiation[7]. Biochemical analysis confirmed a significant increase in protein synthesis in *Nmp4*$^{-/-}$ MSPCs[32]. Sucrose gradient ultracentrifugation was employed to analyze lysates derived from *Nmp4*$^{-/-}$ and WT MSPCs to assess the amounts of translated mRNAs in polysomes[32]. The *Nmp4*$^{-/-}$ MSPC lysates exhibited a significant increase in large polysomes indicating much higher levels of protein synthesis[32]. There was an increase in *Col1a1* mRNA in the largest polysome fraction of the *Nmp4*$^{-/-}$ cells, suggesting enhanced translation of this mRNA[7]. Further biochemical analysis confirmed elevated ribosome biogenesis in the *Nmp4*$^{-/-}$ cells[32]. Consequently, the combination of more *Col1a1* mRNA available for translation, increased amounts of ribosomes, and more efficient *Col1a1* mRNA translation, was consistent with the elevated synthesis of this extracellular matrix protein in the *Nmp4*$^{-/-}$ cells[7]. Furthermore, Seahorse assays revealed that these *Nmp4*$^{-/-}$ MSPCs exhibited an enhanced capacity for glycolytic conversion, a key step in bone anabolism, which is necessary for supporting the high demand of protein synthesis[7].

This enhanced expression of genes driving protein production in *Nmp4*$^{-/-}$ osteogenic cells was accompanied by the upregulation of several UPR genes[7]. The hyper-activation of the UPR in the absence of triggering apoptosis would support the osteoblast high-capacity collagen secretion presumably driving the enhanced response to anabolic PTH.

A UPR and “physiological UPR” briefing.

Key concept:

- The UPR is a stress response that maintains the health of the secreted proteome by (i) temporarily decreasing ER client protein load, (ii) expanding the ER itself, (iii) upregulating protein-folding activity and (iv) promoting misfolded protein degradation.

Before we examine *Nmp4* control of the UPR, we will briefly review here this pathway and its role in secretory cells like osteoblasts. There are several excellent reviews on the UPR[94] and therefore we limit our examination here to the key concepts relevant to the *Nmp4* mechanism of action. Secretory cells including osteoblasts, pancreatic β-cells, pituitary cells, and immune cells have chronic high protein synthesis and delivery requirements. Nascent bone matrix proteins enter the secretory pathway via translocation across the ER membrane into the lumen. Protein folding, other post-translational modifications, and membrane biosynthesis are some of the major activities within this organelle’s lumen[95–97]. Indeed, the ER processes approximately 10,000 different proteins or about 30% of the proteome, thereby accumulating as many as two million client proteins every minute in some secretory cells[95,98]. Binding immunoglobulin protein (BiP, a.k.a. *Hspa5* and *Grp78*) is one of the most highly expressed ER luminal proteins. It is a member of the Hsp70 chaperone family and plays the principal protein folding role in the secretory pathway[95,99].

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The UPR is triggered in response to ER stress, i.e., typically when the ER folding capacity becomes inundated by the sheer bulk of the client load and/or when there is an accumulation of unfolded/misfolded proteins[94]. The activation of the UPR can result in a variety of cellular responses to resolve ER stress, including (i) temporarily diminishing the global rate of protein production, (ii) upregulating lipid membrane biosynthesis to expand the organelle itself, (iii) inducing the synthesis of ER-luminal chaperones, e.g., BiP and (iv) and enhancing the activity of the ER-associated degradation (ERAD) machinery to increase the efficiency of eliminating the recalcitrant misfolded proteins[100]. Apoptosis is also a response option to ER stress and the UPR protein CHOP typically drives this activity[101], although this gene may play a role in the balance between pro-survival and pro-apoptotic states[102].

The details of the UPR mechanics are covered in Iyer and Adams of this Special Issue. Briefly, UPR activation is triggered by changes in BiP association with the luminal portion of one or more of the three sensor ER transmembrane proteins, which monitor the efficiency of protein processing[95]. These three ER stress sensors include two kinases [1] IRE1 (inositol-requiring enzyme 1, a.k.a. ERN1, endoplasmic reticulum to nucleus signaling 1), and [2] PERK (protein kinase RNA-like ER kinase a.k.a. EIF2AK3 eukaryotic translation initiation factor 2 alpha kinase 3) and [3] the transcription factor ATF6 (activating transcription factor 6). Activation of these ER transmembrane proteins mobilizes transcriptional programs that relieve ER stress as described above. Particularly relevant to NMP4, in the absence of its association with BiP, PERK phosphorylates the α-subunit of eIF2 (eukaryotic translation initiation factor 2)[103], which in turn suppresses translation initiation for most mRNAs, thus reducing client protein load on the ER[103]. An exception includes the upregulation of ATF4 (activating transcription factor 4) mRNA translation[103]. ATF4 targets several UPR genes, including GADD34 (growth arrest and DNA damage-inducible protein, a.k.a. Ppp1r15a, protein phosphatase 1, regulatory subunit 15A), which dephosphorylates eIF2α, thus restoring normal protein synthesis, and acting as a negative feedback loop[104]. The altered regulation of GADD34 in *Nmp4*$^{-/-}$ MSPCs may play a significant role in their unique stress response (see below).

The UPR plays functional roles beyond the relief of acute ER stress, and a “physiological UPR” is often activated early in differentiation, particularly in secretory cells that harbor an expansive ER[105–108]. For example, the differentiation of B lymphocytes into antibody-producing plasma cells requires significant ER membrane expansion before any significant protein-client load is observed in the ER[108]. This pre-emptive expansion of the ER depends on activation of the ER stress response pathways, including the UPR, and is required for the secretory phenotype[109]. Nevertheless, how cells ensure that the secretory machinery matches their post-differentiation requirements is not well understood[109].

The “physiological UPR” support of the osteoblast.

Key concept:

- Activation of ER stress and the physiological UPR is a requirement for osteoblastogenesis and is activated during PTH response.

The “physiological UPR” appears to play a role in mediating the high rates of ER client protein synthesis, folding, and secretion in the osteoblast, although the mechanistic

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details remain to be fully elucidated. The PERK pathway plays a key role in osteogenesis and mineralization[110–112], as mice lacking this gene develop severe neonatal osteopenia and diminished matrix mineralization[110]. Mouse *Perk*$^{-/-}$ osteoblasts exhibit a delayed mineralization in culture and diminished expression of Runx2 and osterix, master upstream regulators of osteoblast differentiation[110,111]. Additionally, PERK is required for ATF4 expression, which in turn is necessary for osteoblast differentiation[103,111,113]. The UPR ATF6 signaling pathway plays a significant role in osteoblast differentiation. BMP2 activates ATF6 in MC3T3-E1 osteoblast-like cells, which in turn contributes to osteocalcin expression[114]. OASIS (Old-Astrocyte-Specifically-Induced-Substance) family proteins are UPR proteins that share a region of high sequence homology with ATF6[115]. *Oasis*$^{-/-}$ mice exhibit severe osteopenia caused by a decrease in osteoblast secretion of type I collagen[116].

Although the current FDA-approved osteoanabolics have distinct osteoblast targets, i.e., the PTH receptor (teriparatide and abaloparatide) and sclerostin (romosozumab-aqqg), conscription of the physiological UPR to increase the number of high output osteoblasts without triggering apoptosis might be a common mechanistic feature for the action of these drugs. Several studies provide preliminary data suggesting such a connection[112,117]. The PERK-eif2α-ATF4 signaling pathway plays a role in promoting PTH-mediated osteoblast differentiation[112]. PTH stimulated PERK activity in MC3T3-E1 osteoblast-like cells and in primary mouse calvarial osteoblasts. Inhibiting the PERK-Eif2α-ATF4 pathway significantly diminished mineralization and the expression of numerous proteins that support osteoblast differentiation[112]. Additionally, treatment of these cells with salubrinal, a small molecule that inhibits the phosphatases that dephosphorylate p-eIF2α, enhanced PTH-induced mineralization and osteocalcin secretion[112]. Finally, in a separate study, XBP1 was shown to be transcriptionally activated within 2.5 hrs of PTH treatment in MC3T3-E1 cells[117]. Later related studies determined that the IRE1α-XBP1 pathway not only supports osteoblast differentiation,[118] but that XBP1 transcriptionally supports the expression of the PTH receptor PTH1r[119]. Finally, the PTH anabolic window and treatment plateau for romosozumab-aqqg therapies may represent the limits of the physiological UPR to process the high demands of osteoanabolic-induced bone matrix delivery.

Nmp4 control of the UPR during osteogenesis.

Key concept:

- *Nmp4*$^{-/-}$ MSPCs/osteoblasts in culture exhibit a unique UPR profile consistent with an enhanced secretory capacity.

Ingenuity Pathway Analysis of our bulk RNAseq profiles also predicted that loss of *Nmp4* elevates the action of the UPR pathway while suppressing apoptosis, i.e., a physiological UPR that can sustain high-capacity bone matrix secretion[7]. Indeed, the mRNA expression of several genes of the UPR pathway were strikingly and significantly upregulated in the *Nmp4*$^{-/-}$ MSPCs and osteoblasts in culture[7]. These mRNAs included the key components of the ER protein folding machinery *BiP, Hsp90b1,* and *Hsp47*, the ER stress sensors *Ire1/Xbp1* and *Atf6*, and the trans-acting protein *Atf4* and many other components of this pathway[7,120]. Most interesting was the enhanced mRNA and protein expression of GADD34 in the *Nmp4*$^{-/-}$ cells[7,32]. Recall that this enzyme dephosphorylates eIF2α and

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allows protein synthesis to resume after temporary suspension during pathological ER stress. In the *Nmp4*$^{-/-}$ cells, protein synthesis was sustained during acute tunicamycin-induction of the UPR via a GADD34-mediated dephosphorylation of eIF2α, i.e., the GADD34 negative feedback loop was persistently "on"[32]. These cells also exhibited a significant mRNA increase in the pro-survival factor *Bcl-2*[120], perhaps further supporting the capacity for constant enhanced protein secretion without activating apoptosis. This perpetual GADD34 activity appeared to be a double-edged sword, however, since chronic pharmacological induction of the UPR in *Nmp4*$^{-/-}$ cells decreased cell viability compared to WT cells, but was restored upon inhibition of GADD34 activity[32]. This phenomenon suggests that the *Nmp4*$^{-/-}$ osteogenic cells are at maximum anabolic output and that any further ER stress might be enough to trigger apoptosis.

Conditional removal of *Nmp4* from MSPC/osteoprogenitors, but not later stages of osteogenesis, enhances bone response to anabolic PTH.

Key concepts:

- Results from experiments with *Nmp4* genetically modified mouse models suggest that the potency of an osteoanabolic drug is pre-programmed (and can be re-programmed) in osteoprogenitors but not in later stages of osteogenesis.
- *Nmp4* may also mediate the change in secretory machinery during the osteoblast-to-osteocyte transition.
- In the programming of MSPCs, *Nmp4* may act as a scaling factor, a transcription factor that influences the expression of hundreds of genes governing proteome allocation for establishing secretory capacity in anticipation of high demand.

We conditionally removed *Nmp4* from cells at different stages of osteogenic differentiation. *Nmp4*-floxed (*Nmp4*$^{fl/fl}$) mice were crossed with one of three Cre-driver mice that express Cre-recombinase in either (i) long bone MSPCs but not spine MSPCs (*Prx1Cre*+), (ii) mature osteocalcin-expressing osteoblasts (*BglapCre*+), or (iii) transitional osteocytes (*Dmp1Cre*+)[31]. These mice, along with their Cre- (WT) controls were treated with PTH or vehicle control from 10 weeks to 14 weeks of age (4 weeks therapy) or to 17 weeks of age (7 weeks therapy). *Nmp4*$^{fl/fl}$*;Prx1Cre*+ mice largely phenocopied the global *Nmp4*$^{-/-}$ skeleton i.e., they exhibited a significantly enhanced PTH-induced increase in femur trabecular bone volume/total volume (BV/TV) compared with the *Nmp4*$^{fl/fl}$*;Prx1Cre*- controls at 4 weeks therapy that further exceeded bone formation of the Cre- mice at 7 weeks treatment[31]. The spine did not show an enhanced response to PTH therapy, as expected since *Prrx1* is not expressed in vertebral bone[31,121], which permits the use of the spine as an internal control. This boosted response to PTH was coincident with enhanced bone formation with no evidence of changes in bone resorption, as observed with the global *Nmp4*$^{-/-}$ mice[12,31]. However, conditional loss of *Nmp4* from the osteocalcin-expressing osteoblasts (*Nmp4*$^{fl/fl}$*;BglapCre*+) showed no enhanced response to hormone treatment[31]. Finally, conditional removal of *Nmp4* in osteocytes (*Nmp4*$^{fl/fl}$*;Dmp1Cre*+) increased femoral and spine BV/TV without boosting response to PTH[31].

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Does *Nmp4* influence the establishment of the osteocyte secretory machinery during the osteoblast-to-osteocyte transition, and does it regulate the osteocyte secretome? As the osteoblast switches from an early osteocyte and then again to a mature osteocyte state, there are patent changes in the cell secretory ultrastructure and morphology[122,123]. These changes include a reduction in overall cell size while the nucleus-to-cytoplasm ratio increases. The number of organelles decrease including a reduction in the size/extent of the ER and Golgi as well as fewer mitochondria[122,123]. Regarding the osteocyte secretome, these cells regulate bone anabolism by the release of factors that increase (e.g., PGE2, IGF-1, Wnts) or limit (e.g., sclerostin, DKK1) osteoblast-mediated bone formation[124]. Intermittent PTH diminishes osteocyte secretion of sclerostin, thus increasing bone formation, and increases their release of RANKL to enhance osteoclastogenesis, the delayed phase of the anabolic response[125]. The mature osteocyte is the primary mechanosensor of the skeleton and senses the micromechanical environment via changes in oscillatory fluid flow-induced shear stress[126]. Mechanically activated osteocytes can enhance the bone formation capacity of osteoblasts by cell contact or by the release of anabolic factors[126]. This osteocyte secretome also enhances MSPC proliferation, recruitment, and differentiation and recent evidence suggests that released osteocyte extracellular vesicles carry some of these anabolic cues[127]. Additionally, physical interactions between osteocytes, osteoprogenitors, and osteoblasts mediated by gap junctions may play a role in this interesting phenotype[128,129]. Perhaps the $Nmp4^{-/-}$ osteocytes of the $Nmp4^{fl/fl}$;*Dmp1Cre*+ mice and the $Nmp4^{fl/fl}$;*Prx1Cre*+ mice release a strengthened anabolic signal. In this hypothetical scenario, this augmented anabolic secretome activates the hyper-reactive $Nmp4^{-/-}$ MSPCs/osteoblasts in the $Nmp4^{fl/fl}$;*Prx1Cre*+ mice and the less responsive $Nmp4^{+/+}$ MSPCs/osteoblasts in the $Nmp4^{fl/fl}$;*Dmp1Cre*+ animals. Indeed, the $Nmp4^{fl/fl}$;*Prx1Cre*+ mice harbor fewer osteoprogenitors than their $Nmp4^{fl/fl}$;*Prx1Cre*- controls after 7 weeks of PTH therapy, perhaps because of an accelerated differentiation into osteoblasts, but no such difference between the $Nmp4^{fl/fl}$;*Dmp1Cre*+ mice and their Cre- controls was observed[31]. This finding might explain the increased bone formation in the $Nmp4^{fl/fl}$;*Dmp1Cre*+ without the enhanced response to hormone[31]

As described in the Introduction, scaling factors are evolutionary conserved trans-acting proteins that influence the expression of hundreds to thousands of genes that *in toto* establish the secretion machinery and thus the capacity of secretory cells[23–30,130]. MIST1 and CREB3L2 are the most thoroughly investigated scaling factors. These master regulators of secretory infrastructure cooperate with the UPR transcription factor XBP1 to regulate complex networks of genes involved in ribosome biogenesis, tRNA charging, the physiological UPR, and the metabolic activity necessary to support high output protein production and delivery[26–29,131,132]. Indeed, like MIST1 and CREB3L2, *Nmp4* has a broad influence over these very same regulatory networks but appears to limit or cap the ultimate secretory capacity of bone cells instead of promoting its expansion[7]. The assembly of the cell's secretory machinery would be expected to occur at a point in differentiation before the onset of demand. This appears to be the case in the differentiation of B lymphocytes to plasma cells[108]. These lymphocytes progress through the stages of transitional preplasmablasts (prePBs), a proliferative cell population, which in turn further differentiate into plasmablasts (PBs)[133]. The PBs ultimately give rise to the antibody-

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secreting plasma cell, which produces large quantities of Ig chains[134]. It is during the prePB to PB transition that genes involved in protein production and delivery are upregulated in the B lymphocyte transcriptome, i.e., before the onset of high demand for Ig chain secretion[134].

We propose a model for *Nmp4* control of osteoanabolic efficacy that integrates data from the genetically modified mouse models and whole-genome ChIPseq, transcriptomic, and biochemical analyses[7–12,31,32]. In this scenario, the amount of new bone formed in response to an osteoanabolic drug is programmed in the MSPCs/osteoprogenitors. This includes *Nmp4* acting as a scaling factor that sets the ceiling for matrix production and secretion in anticipation of the high demand required for osteoblast-mediated bone formation. The *Nmp4*$^{-/-}$, Prx1-expressing MSPCs are re-programmed which includes a unique stress response linked to the UPR resulting in the establishment of an expanded secretory capacity supporting an exaggerated response to an osteoanabolic drug without triggering osteoblast apoptosis. A similar pre-programming may occur early in the osteoblast-to-osteocyte transition (Figure 3).

Nmp4-mediated control of secretion is not limited to bone.

Key concepts:

- Loss of *Nmp4* attenuates pancreatic β-cell insulin secretion. Despite this defect, global *Nmp4*$^{-/-}$ mice are less sensitive to high fat diet-induced weight gain, increases in % fat mass, and reductions in glucose tolerance and insulin sensitivity.
- Loss of *Nmp4* diminishes the release of cytokines and chemokines from lung epithelial cells during influenza A virus (IAV) infection, however, global *Nmp4*$^{-/-}$ mice are resistant to IAV morbidity. Similarly, loss of *Nmp4* significantly reduces the development of arthritis, in part through attenuated release of key secretory molecules.

Pancreatic β-cells—Loss of *Nmp4* affects the phenotypes of secretory cells in non-osseous tissues, including pancreatic β-cells[13]. *Nmp4* supports pancreatic β cell development and insulin secretion, unlike its suppression of osteoblast secretion[13]. Based on our data showing that loss of *Nmp4* enhanced osteoblast secretion of bone matrix, we had predicted that insulin secretion would be elevated in *Nmp4*$^{-/-}$ pancreatic β-cells. Unexpectedly, these global knockout mice exhibited deficits in baseline pancreatic β-cell function[13]. Specifically, immunohistochemical analysis of pancreas sections from 8-week-old mice revealed a near significant reduction in β-cell mass in the *Nmp4*$^{-/-}$ mice[13]. Additionally, glucose stimulated insulin secretion (GSIS) was significantly reduced in islets isolated from *Nmp4*$^{-/-}$ mice compared to the WT islets. Consistent with these *ex vivo* assays, global *Nmp4*$^{-/-}$ mice displayed decreased circulating insulin levels compared to WT controls[13]. These data demonstrated that *Nmp4* supports β-cell secretory function and does not suppress this mechanism as it does in osteoblasts.

Intriguingly, even with the β-cell deficits, the global *Nmp4*$^{-/-}$ mice were less sensitive to HFD-induced weight gain, increases in % fat mass, and reductions in glucose tolerance and insulin sensitivity[13]. The HFD did not further impair the diminished GSIS seen in

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the *Nmp4*$^{-/-}$ mice but significantly decreased this response in the WT HFD cohort[13]. Based on the data from this study, we concluded that although *Nmp4* supports pancreatic β-cell function it also suppresses peripheral glucose utilization, perhaps contributing to its restraint of induced bone formation. Disabling of *Nmp4* in select peripheral tissues and/or secretory cells may provide a strategy for enhancing both induced osteoanabolism and energy metabolism in patients comorbid for osteoporosis and type 2 diabetes.

Lung epithelial cells and macrophages—In addition to the above effects in the endocrine system, *Nmp4* regulates the secretory phenotypes of cells involved in the immune response to influenza A virus (IAV) infection[14]. Human-associated influenza viruses have a predominant affinity for the lung secretory cells[135]. Upon infection, the lung secretory cells increase the expression of hundreds of interferon (IFN)-stimulated genes (ISGs), but also release several cytokines and chemokines[135]. The ISGs hinder viral replication, whereas the release of cytokines and chemokines by the infected secretory cells recruit and activate neutrophils and monocytes to initiate the local lung inflammatory response of the innate immune system[135]. IAV infection presents the immune system with the significant challenge of balancing the functions of (i) limiting pathogen spread vs. (ii) constraining self-inflicted inflammation-mediated tissue damage[136]. Severe outcomes of IAV typically result from deficits in the execution of one or both of these tasks[136].

Nmp4 supports the release of cytokines and chemokines from lung epithelial cells during IAV infection[14]. Indeed, global *Nmp4*$^{-/-}$ mice were protected from this pathogen, losing only 5% body weight compared to a 20% loss in the WT cohorts, but this was not due to viral clearance or CD8+T/CD4+ T cell or humoral responses[14]. Instead, loss of *Nmp4* reduced the recruitment of monocytes and neutrophils to the lungs of infected mice[14]. Consistent with this observation there was significantly diminished expression of the chemokines *Ccl2*, *Ccl7*, and *Cxcl1* and the pro-inflammatory cytokines *Il1b* and *Il6* in infected lung[14]. A key finding was that the neutrophil and monocyte levels in the circulation and bone marrow were not different between the genotypes, thus indicating that a primary function of *Nmp4* is to regulate chemokine-driven intrapulmonary leukocyte recruitment during IAV infection. We determined that *Nmp4* transcriptionally drives the chemokine genes and controls their expression in both lung epithelial cells and macrophages. These results identify a key role for *Nmp4* in driving the inflammatory recruitment of neutrophils and monocytes to the infected lung. We propose that the diminished secretory response of *Nmp4*$^{-/-}$ lung epithelial cells and macrophages helps to constrain the self-inflicted inflammation-mediated tissue damage without significantly increasing pathogen spread, thus moderating disease severity.

Chondrocytes—Our IAV study is consistent with an earlier investigation showing that *Nmp4* drives *Il1b* transcription and the development of arthritis[15]. Nakamoto et al. interrogated the pathological roles of *Nmp4* in this disease using the K/BxN serum transfer model. The investigators reported that NMP4 protein was expressed in the articular chondrocytes of healthy mice at low levels and its expression was increased when arthritis was induced[15]. Arthritis induction resulted in joint swelling and redness in *Nmp4*$^{+/+}$ mice but not the *Nmp4*$^{-/-}$ mice. The *Nmp4*$^{-/-}$ mice exhibited a reduced invasion of inflammatory

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cells in joint tissue similar to what we later reported in the lung for the IAV study. Quantitative PCR analyses of mRNA from joints demonstrated that the arthritis-induced increase in expression of *Il1b* was suppressed in the global *Nmp4*$^{-/-}$ mouse. NMP4 was shown to bind to the *Il1b* promoter and activate and drive its transcription in ST2 cells[15]. The investigators concluded that *Nmp4* plays a role in the development of arthritis at least in part through regulation of key secretory molecules related to this pathogenesis[15].

Is there a global mechanism for *Nmp4* secretory control?

Key concepts:

- The *Nmp4*$^{-/-}$ mouse phenotype is most prominent upon an increase in secretory demand, whether it be in response to an osteoanabolic drug, metabolic challenge, or viral pathogen infection, suggesting that loss of *Nmp4* results in a modified stress response in secretory cells.
- The tissue-specific differences in *Nmp4* secretory control could originate in part from this protein's context-specific effects on transcription and its role in chromatin organization.

Are the observed differences of *Nmp4* status on cell secretory activity in various tissues the result of local modifications on a uniform mechanism of action? We have the most information about the *Nmp4*-mediated control of phenotype from bone cells where we have ChiPseq, and bulk RNAseq, as well as phenotypic anchoring of the transcriptional data through analysis of cell metabolism, protein synthesis, secretion, bone material properties[7,8,31,32]. Therefore, mechanistic generalizations will be made within this context.

To date, the *Nmp4*$^{-/-}$ mouse phenotype is most prominent upon an increase in secretory demand, whether it be in response to an osteoanabolic drug, metabolic challenge, or viral pathogen infection, suggesting that unmasking the phenotype requires a provocation or insult. This suggests that a major role for *Nmp4* is pre-setting protein production and secretory capacity, likely early in cell differentiation when the secretory infrastructure is under formation. This hypothesis is consistent with the osteogenic *Nmp4* conditional knockout studies[31]. *Nmp4* appears to be less significant in regulating the basal activities of these functions, as *Nmp4*$^{-/-}$ mice under normal vivarium conditions and not challenged with a particular stress appear generally healthy and vigorous[9].

The tissue-specific differences in *Nmp4* secretory control could originate in part from this protein's context-specific effects on transcription[7,83] and its regulation of chromatin organization and modification[8]. As described above, NMP4 harbors two trans-activation domains whose activities are sensitive to allosteric effects induced upon zinc finger association with the variant AT-rich consensus site[83]. Combining our RNAseq and CHiPseq data sets from our bone studies showed that approximately 28% of the genes occupied by NMP4 exhibited a significant increase in expression upon loss of this trans-acting protein during osteogenesis, indicating gene repression by NMP4[7,8]. However, about 9% of the genes showed a decrease in expression in the *Nmp4*$^{-/-}$ cells, suggesting that NMP4 directly enhances the expression of distinct target genes in the same cells[7,8]. This differential influence on gene expression is similar to the allosteric effect on the transcription factor

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Pit-1 binding to the growth hormone promoter in somatotropes where it activates target genes but represses genes in lactotopes[87]. The difference in Pit-1 transcriptional activity depends on a two-base pair spacing in accommodation of the bipartite POU domains located on the growth hormone promoter site[87]. The allosteric effects of the growth hormone promoter binding element on the configuration of Pit-1 serve as one of the critical determinants of its association with the corepressor machinery in the appropriate cellular context[87]. Thus, allosteric modulation of NMP4 may expand its transcriptional repertoire in the context of secretory control.

As part of the NMP4 CHiPseq analysis of MC3T3-E1 osteoblast-like cells, we used the ENCODE ChIP-seq Significance Tool[137] to probe existing datasets for enriched transcription factors within our *Nmp4* core target gene list[8,93]. This analysis determined that NMP4 binding to its target genes' promoters principally co-occurs with proteins that regulate chromatin organization and loop formation including CTCF, CHD2, GCN5, SIN3A, and HCFC1[8]. CHD2 is a chromatin remodeler involved in cell fate decision and has an AT-rich consensus sequence like NMP4[138]. GCN5 is a histone acetyltransferase coactivator[139], SIN3A provides a scaffold that interacts with numerous factors associated with chromatin-mediated transcriptional silencing[140], and HCFC1 is a chromatin-associated protein that can activate and repress transcription by coupling select histone-modifying enzymes to transcription factors[141,142]. Therefore, tissue-specific differences in chromatin organization could also contribute to NMP4 control of secretory phenotype. Consistent with this hypothesis is the recent study showing that target gene selection of the UPR sensor ATF6 is significantly influenced by epigenetic modifications[143].

Summary And Knowledge Gaps

Why osteoanabolics rapidly lose their therapeutic efficacy represents a major knowledge gap in the treatment of osteoporosis. The likely ultimate barrier to improving these drugs in the treatment of severe osteoporosis is accommodating the pharmacological increase in matrix production and expansion of the osteoblast secretory capacity without triggering apoptosis. Elevated secretion of bone matrix burdens osteoblasts with ER stress, which if unresolved, will lead to cell death. The osteoblast differentiation program is linked to the physiological UPR to raise the ER secretory capacity of the mature cell in a preemptive adaptation to high anabolic output.

ChIPseq and bulk RNAseq data showed that *Nmp4* influences over 200 pathways in MSPCs. Phenotypic anchoring of these genomic and transcriptomic data through evaluation of protein synthesis, secretion, UPR profiling, cell metabolism, bone material properties, and experiments with global $Nmp4^{-/-}$ and conditional knockout mice has led to our hypothesis that $Nmp4^{-/-}$, Prx1-expressing MSPCS are re-programmed with a unique stress response driving the enhanced potency of anabolic PTH. The proposed role as a scaling factor may support *Nmp4* unilateral influence one the expression of hundreds to thousands of genes comprising the regulatory networks responsible for elaborating the secretory infrastructure. This permits the $Nmp4^{-/-}$ osteoblast to handle large ER client protein loads without succumbing to apoptosis, the proposed barrier to osteoanabolic efficacy.

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Several questions remain to be answered. What are the key *Nmp4* target MSPCs pathways that pre-program bone response to osteoanabolics? Does this program link lineage commitment, protein production, and secretory capacity via a novel physiological UPR? What triggers this unique stress response? How are these putative pathways differentially regulated during the osteoblast-to-osteocyte transition? Are these pathways responsive to anabolic mechanical loading? Does sex impact Nmp4 control of bone response to osteoanabolics?

Secretory cells play a significant role in mediating the crosstalk between bone and several of the body's systems, and *Nmp4* appears to influence the phenotype of these kinds of cells in osseous as well as non-osseous tissues. These findings place *Nmp4* in a unique position for influencing the communication between bone and several other secretory tissues. *Nmp4* may be a component of a general mechanism for setting the capacity of secretory cell protein production and delivery in response to pharmacological or physiological challenges. Its role in either limiting or expanding secretory competence is tissue-specific, which is perhaps a consequence of its influence on chromatin organization or the action of its variable AT-rich consensus sequence as an allosteric ligand. Elucidating how *Nmp4* arbitrates the quantity and secretory capacity of bone cells and other tissues are significant gaps in our knowledge and when successfully addressed will improve our understanding of how the limits of cell secretion are established and provide novel strategies for clinically improving osteoanabolic efficacy and manipulating the crosstalk between bone and non-osseous systems.

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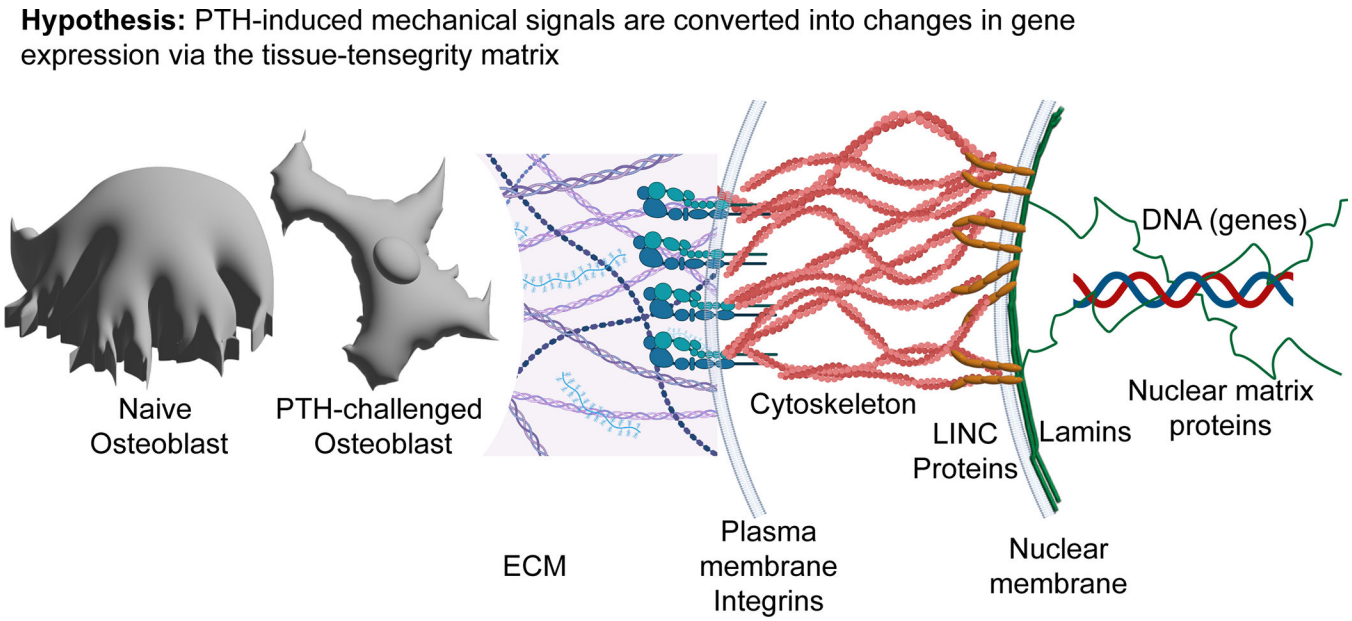

Figure 1:

A conceptual framework based on the tissue-tensegrity-matrix model[66–69] to integrate anabolic PTH-induced changes in osteoblast morphology with changes in gene expression[80]. In this paradigm the genome is literally "hard-wired" to the sub-structure of the adherent cell, i.e., there are physical links between the extracellular matrix, integrin receptors, the cytoskeleton, LINC proteins, and the nuclear matrix which, in turn, makes connections to the DNA. Nuclear matrix proteins and nuclear matrix-associated proteins can bind to or near the regulatory elements of genes and some are responsive to extranuclear mechanical signals[73]. These proteins thus play significant roles in the regulation of gene expression via mechanotransduction[73]. Figure generated with BioRender (https://biorender.com/).

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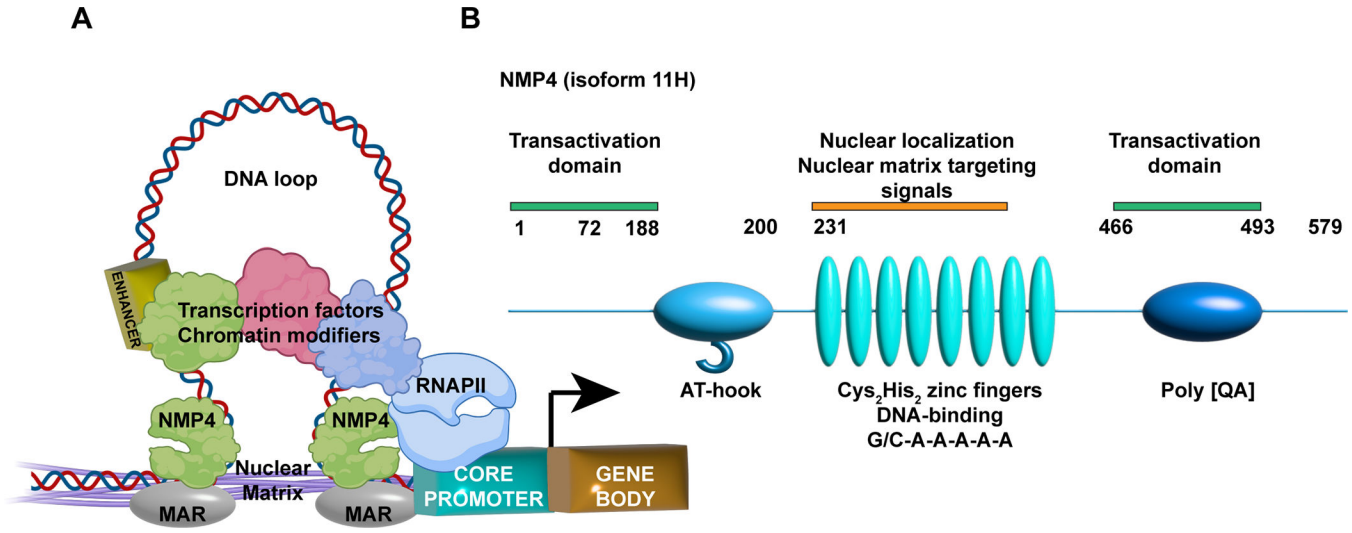

Figure 2:

[A] NMP4 is a nuclear matrix, MAR-binding protein, nuclear matrix architectural transcription factor. The graphic representation illustrates its proposed role in influencing gene expression as a MAR-binding protein. The DNA-bending capacity of NMP4[2] may alter the interactions between transcription factors on distant enhancers and trans-acting proteins of the target promoters[78]. ChIPseq analysis determined that NMP4 binding to its target gene promoters co-occurs with proteins that regulate chromatin organization and loop formation[8]. **[B]** Schematic of NMP4 isoform 11H[6,83]. NMP4 contains a well-defined DNA binding domain (Cys_2His_2 zinc fingers), two distinct transactivation domains located in the N- and C-termini, and an AT-hook domain[6,83]. The zinc fingers bind to the minor groove of the AT-rich consensus site likely recognizing the local structural contour instead of the variable nucleotide sequences presented in the major groove a.k.a. 'indirect readout'. This homopolymeric dA•dT binding site might act as an allosteric ligand conferring context-specific/site specific functionality to the transactivation domains. The AT-hook may also associate with the minor groove of AT-rich consensus site and mediate the observed NMP4 DNA bending (see text for detail). Figure generated with BioRender (https://biorender.com/).

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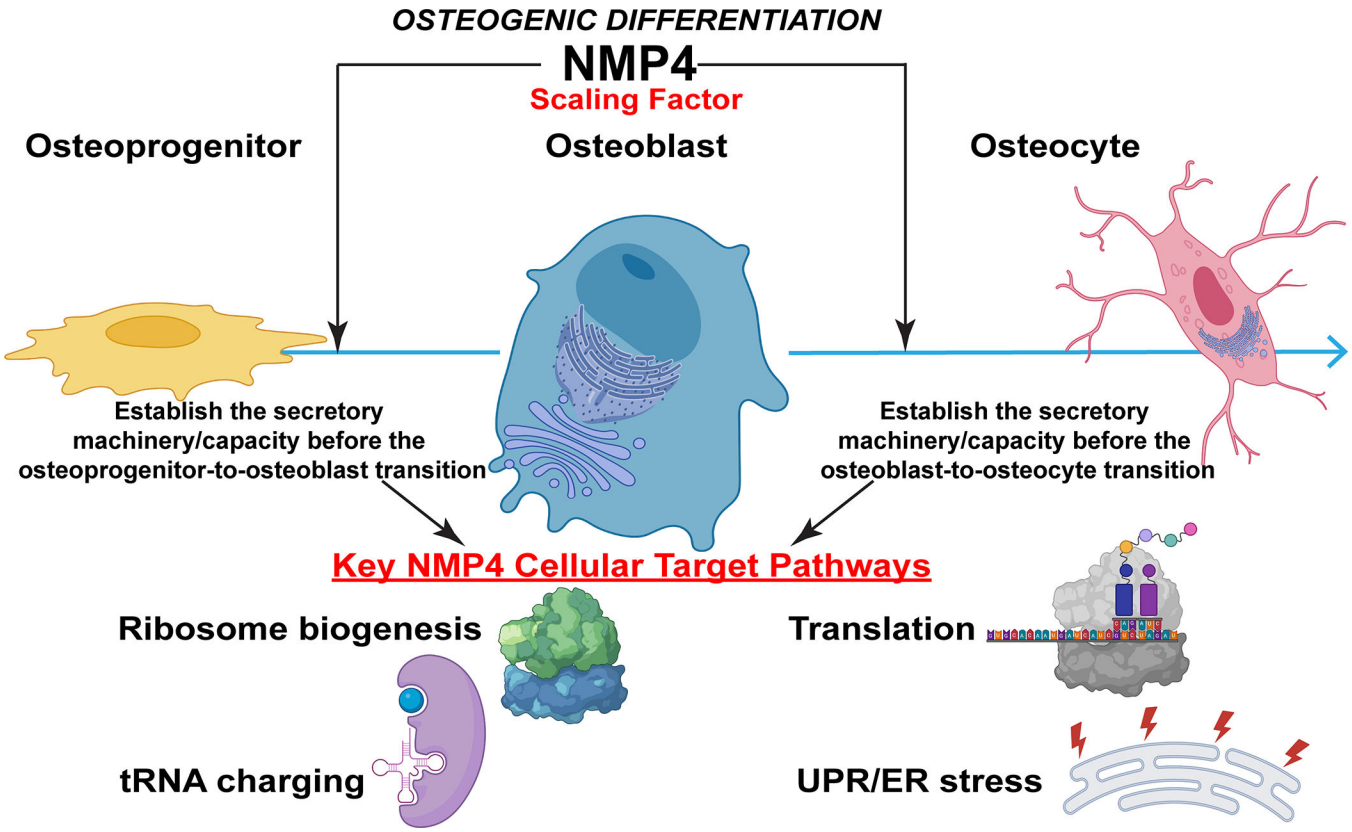

Figure 3:

The proposed role for NMP4 as a scaling factor that sets the secretory capacity of osteoblasts and osteocytes. Early in differentiation, NMP4 acts to limit the expansion of the secretory machinery of the osteoblast by regulating the expression of hundreds of genes that comprise pathways controlling protein production and delivery. These genes include those involved in ribosome biogenesis, tRNA charging, translation, and the physiological UPR[7,31,32]. NMP4-mediated control of genome function includes its gene- and site-specific interactions with corepressors and coactivator (see Figure 2). The timing of the NMP4-mediated establishment of bone cell secretory capacity is critical and must occur before the osteoblast reaches maturity[31]. Some of the mature osteoblasts proceed to differentiate into osteocytes, once again requiring a significant change in the organization of the secretory organelles that is mediated by NMP4. This mechanism may be the ultimate barrier to osteoanabolic efficacy. Figure generated with BioRender (https://biorender.com/).