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## Building and maintaining joints by exquisite local control of cell fate

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### Abstract

We owe the flexibility of our bodies to sophisticated articulations between bones. Establishment of these joints requires the integration of multiple tissue types: permanent cartilage that cushions the articulating bones, synovial membranes that enclose a lubricating fluid-filled cavity, and a fibrous capsule and ligaments that provide structural support. Positioning the prospective joint region involves establishment of an “interzone” region of joint progenitor cells within a nascent cartilage condensation, which is achieved through the interplay of activators and inhibitors of multiple developmental signaling pathways. Within the interzone, tight regulation of BMP and TGF $\beta$  signaling prevents the hypertrophic maturation of joint chondrocytes, in part through downstream transcriptional repressors and epigenetic modulators. Synovial cells then acquire further specializations through expression of genes that promote lubrication, as well as the formation of complex structures such as cavities and entheses. Whereas genetic investigations in mice and humans have uncovered a number of regulators of joint development and homeostasis, recent work in zebrafish offers a complementary reductionist approach toward understanding joint positioning and the regulation of chondrocyte fate at joints. The complexity of building and maintaining joints may help explain why there are still few treatments for osteoarthritis, one of the most common diseases in the human population. A major challenge will be to understand how developmental abnormalities in joint structure, as well as postnatal roles for developmental genes in joint homeostasis, contribute to birth defects and degenerative diseases of joints.

### INTRODUCTION

Joints connect neighboring bones within the vertebrate skeleton. Fibrous joints called sutures separate the flat bones of the skull and allow for flexibility during childbirth (Figure 1(a)). Cartilaginous joints, for example between the vertebrae, provide limited mobility and act as shock absorbers (Figure 1(b)). The most sophisticated types of joints are the lubricated synovial articulations, such as those of the highly mobile limbs and jaw (Figure 1(c)). Despite their differing structures and extents of mobility, all three types of joints share key features. For example, developing joints are enriched for skeletal progenitor cells, which in the case of sutures provide a continuing supply of osteoblasts for bone growth.<sup>1–5</sup> Conversely, each joint is characterized by its own specialized morphology and function,

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which is reflected by unique patterns of gene expression by their resident skeletal and supporting cells. A critical early event in the creation of all joints is the suppression of osteoblast or chondrocyte differentiation at particular sites within the developing skeleton. For many synovial joints, suppression of chondrocyte maturation occurs within a broad region called the interzone.<sup>6,7</sup> Lineage analysis demonstrates that interzone cells contribute not only to the articular cartilage lining the joint cavity but also to the synovium, menisci, and ligaments.<sup>8,9</sup> While much remains to be learned about how joints are positioned, the precise spatial deployment of activators and inhibitors of diverse signaling pathways is a recurrent theme. One consequence of such signaling is the induction of several types of transcription factors and chromatin modifiers, which function to inhibit cartilage maturation within the interzone. What is less clear is how later events in joint development are regulated, such as production of the lubricating protein PRG4/Lubricin by articular chondrocytes,<sup>10,11</sup> creation of the joint cavity,<sup>12</sup> and the local differentiation of interzone cells into menisci and ligament attachment points (entheses).

While major congenital anomalies of joints are uncommon, the progressive loss of joint structure and function in osteoarthritis is the leading cause of disability in the United States.<sup>13</sup> Genetic factors contribute substantially to osteoarthritis (e.g., estimated at 60% for the hip<sup>14</sup>), with recent studies beginning to uncover specific loci linked to arthritis predisposition.<sup>15</sup> An emerging theme is that minor defects in developmental morphogenesis may lead to architectural defects in postnatal joints,<sup>16</sup> which result in the increased wear-and-tear that predisposes to osteoarthritis. A key feature of osteoarthritis is the ectopic hypertrophic differentiation of articular chondrocytes at the joint surface, which erodes the cartilage cushions protecting the bones and eventually the bones themselves.<sup>17</sup> This pathological observation suggests that a challenge for prolonged joint health is preventing joint-lining chondrocytes from undergoing hypertrophic maturation. Interestingly, many of the same signaling pathways and transcription factors that help to establish articular chondrocyte fate continue to be expressed at joints into adulthood, with postnatal genetic manipulations in some cases establishing continued requirements in maintaining articular fate.<sup>9,18</sup> There are also potential roles for epigenetic modifications in establishing long-term repression of the hypertrophic program in articular chondrocytes,<sup>19</sup> as well as other factors selectively required for the maintenance but not development of joints.

## USE OF ZEBRAFISH FOR DEVELOPMENTAL STUDIES OF JOINTS

Although most studies on joints have been conducted in mammals, the zebrafish has recently emerged as an alternative model to investigate basic joint biology. Zebrafish have many of the same types of joints found in mammals (Figures 1 and 2). Sutures connect the skull bones in both fish and mammals, with undifferentiated mesenchyme residing between the interleaved bony plates<sup>20</sup> (Figures 1(a) and 2(c) and (d)). Imaging studies in living zebrafish have revealed the stepwise formation of wild-type sutures, as well as abnormal suture development upon genetic or pharmacological perturbations (e.g., loss of retinoic acid signaling).<sup>21–23</sup> Zebrafish also have intervertebral discs that are prone to degeneration upon aging.<sup>24</sup> However, the discs of adult zebrafish differ in structure from those in mammals in lacking cartilage, with histology showing adipocytes between the vertebral bones rather than nucleus pulposus and annulus fibrosus structures as in mammals<sup>25</sup> (Figures 1(b) and 2(c)

and (d)). In the zebrafish fry, two cartilaginous joints in the head have been studied extensively: the jaw joint between the Meckel's and palatoquadrate cartilages and the bipartite hyoid joint between the ceratohyal, interhyal, and hyosymplectic cartilages (Figure 2(a)). Studies of these joints have revealed conserved roles for GDF family ligands<sup>26</sup> and new roles for Nkx3,<sup>27</sup> and Irx family<sup>28</sup> transcription factors. Contrary to previous dogma, a recent study also suggests that fishes have synovial-like lubricating joints, particularly in their jaw and fins.<sup>25</sup> Building on older histological observations,<sup>29</sup> this study showed that the jaw and fin joints of diverse fish species (zebrafish, stickleback, and spotted gar) have many of the features of mammalian synovial joints including articular cartilage, a joint cavity and synovium (Figures 1(c) and 2 (c) and (d)). Best illustrated in the much larger gar, the fish jaw joint also displays the same multilayered articular cartilage as in mammalian synovial joints (Figures 1(c) and 5(a)): a superficial zone of flattened chondrocytes, a deeper transitional zone of rounder chondrocytes, a radial zone of stacked chondrocytes, and finally calcified chondrocytes that integrate into the underlying bone (Figure 5(b)). Although it was not demonstrated that the synovial capsule is fully enclosed, the jaw joints of these fishes exhibited a clear synovial fibroblast layer at the periphery and an external fibrous capsule. At a molecular level, the jaw joints of all three fishes, as well as the pectoral fin joints of zebrafish (Figure 2(c)), express homologs of the lubricant proteoglycan PRG4/Lubricin,<sup>25</sup> a prominent marker of superficial chondrocytes and synovium at mammalian joints.<sup>11</sup> Furthermore, zebrafish lacking the Lubricin-encoding gene *prg4b* developed the same arthritic changes to their jaw and fin joints as observed in mice and human patients lacking Lubricin.<sup>11,25,30</sup> These findings show that synovial joints likely evolved at least in the common ancestor of all bony vertebrates, and that zebrafish are a relevant model for the study of arthritis. While it remains unclear the extent to which the jaw and related joints of fishes have all the properties of mammalian synovial joints, future work in zebrafish has the potential to provide new insights into synovial joint development and disease. Where appropriate, we highlight recent contributions of the zebrafish model to our understanding of joint biology and pathology.

## POSITIONING THE JOINT DOMAIN

The future joint domain could be determined in at least two major ways (Figure 3). First, an initial chondrogenic condensation can be split into two or more distinct zones by creation of a morphologically distinct interzone region, which is characterized by flattened and highly compacted cells relative to the adjacent growth plates. An example of this mechanism is the creation of the interphalangeal joints in the digits. Second, the prospective joint domain can be created by the appositional growth of two neighboring condensations, such as what occurs between the anlagen of the femur and pelvic bone<sup>31</sup> and for the temporomandibular joint of the mammalian jaw.<sup>32</sup> Interestingly, compromised condensation formation in *barx1* zebrafish mutants can lead to ectopic joints, potentially through aberrant condensation splitting.<sup>33</sup> It remains unclear, however, what effects the mode of joint specification have on later development. In principle, the preservation of progenitor zones at the leading edges of appositionally growing condensations may result in their later fusion into a structure closely resembling an interzone.

## Reaction–Diffusion Mechanisms of Joint Spacing in Fin and Limb Skeletons

In many cases, joints develop in a periodic pattern, e.g., the interphalangeal joints of the digits. Studies of the ray joints of the zebrafish fins have begun to uncover potential mechanisms of iterative joint specification. The proximal portion of the fish fin is thought to be homologous to tetrapod limbs,<sup>34</sup> with the more derived distal portion containing a series of bony rays segmented at regular intervals by fibrous joints (Figure 4). While these ray joints differ from the synovial-like joints at the base of the fin, they have been useful for understanding how repeated joints can be established. Loss-of-function mutations in the gap junction protein-encoding gene *connexin43* (*short-fin*) result in joints spaced closer together, independent of effects on fin size, with Connexin43 overexpression eliminating joints.<sup>35,36</sup> Gain-of-function mutations in the potassium channel-encoding gene *kcnk5b* (*another-long-fin*) also result in fins with irregularly spaced joints.<sup>37</sup> In addition, the even-skipped transcription factor *evx1* is essential for ray joint development in zebrafish.<sup>38–40</sup> While it is unclear the extent to which iterative patterning of ray joints would be relevant to mammalian joint patterning, mesenchymal cells within the interzone of developing mammalian joints do express Connexins 32, 40, and 43,<sup>12,41</sup> and *Connexin40*<sup>−/−</sup> mice display losses of specific joints in the distal limbs.<sup>42</sup> Mutations of *CONNEXIN43* in Oculodentodigital Dysplasia syndrome also result in skeletal defects, including missing and fused phalanges.<sup>43</sup> The conserved requirement of Connexins in fish and mammals suggest that gap junctions could be required for the efficient spread of some unknown small molecule between cells in both species, although most growth factors would be assumed to be too large to pass through these channels. Nonetheless, modeling studies in zebrafish reveal how the expression and diffusion of just two factors is sufficient to explain the repeated spacing of joints within the fin.<sup>44</sup> At the tip of the elongating fin, a growth factor would promote mesenchymal proliferation and inhibit joints, with joint formation being induced when the concentration of the growth factor falls below a certain threshold. At this position, a joint-promoting factor would be induced, which would secondarily inhibit other joints from forming nearby. Only in positions where the two factors were below a critical threshold would a new joint form (Figure 4(b)). It is tempting to speculate that a similar mechanism might occur for joints in the hands and feet of tetrapods, such as the periodically spaced Phalangeal joints of the digits. Good candidates for the limb/fin growth factor are FGFs, which are produced by the apical ectodermal ridge and required for limb/fin outgrowth.<sup>45</sup> WNT9a/14 is a candidate joint factor that would inhibit additional joints at a distance, as its misexpression in mouse induces an ectopic joint while inhibiting development of the adjacent normal joint.<sup>46</sup> Variations on the theme of diffusible activators and inhibitors generating repeated patterns are common in biological systems. Such reaction–diffusion mechanisms proposed by Turing<sup>47</sup> have been used to explain the patterning of teeth,<sup>48</sup> ectodermal appendages,<sup>49</sup> and skin pigmentation.<sup>50</sup> Future work is needed to explore the relative contribution of prepatterning versus iterative activator–inhibitor interactions for the positioning of different joints.

## Developmental Signaling to Establish the Interzone of the Future Jaw Joint

In contrast to the repeated joints in appendages, there are other contexts where the joint interzone appears to be prespecified by the convergence of multiple developmental signaling pathways. An illustrative example is the zebrafish jaw joint, which is positioned by

integration of Edn1 and Bmp signaling within the neural crest-derived precursors of the jaw skeleton.<sup>51</sup> In zebrafish, loss of the Edn1 ligand or its Ednra receptors results in defects in the lower jaw and jaw joint.<sup>52,53</sup> The joint appears to be particularly dependent on optimal Edn1 signaling as partial reductions of the pathway in *furina*, *plcb3*, or *mef2ca* mutants have greater effects on joint versus lower jaw development.<sup>54–56</sup> An important target of Edn1 signaling in joint formation is the transcription factor *nkx3.2/bapx1*, as antisense reduction in zebrafish results in specific loss of the jaw joint.<sup>27</sup> In chick, ectopic expression of BMP4 and FGF8 abrogates *Nkx3.2* expression and results in loss of joint structures such as the retroarticular process.<sup>57,58</sup> In zebrafish, Bmp4 misexpression or reduction of the Bmp antagonist Gremlin2 disrupts jaw joint formation, with Edn1 and Jagged-Notch signaling functioning together to confine *grem2b* expression to nascent facial joints.<sup>28,59</sup> Bmp signaling restricts the jaw joint-forming domain in part through induction of Hand2, which prevents expansion of *nkx3.2* expression into the lower jaw.<sup>27</sup> In humans, mutations in *EDN1* or its signaling components such as *PLCB4* and *GNAI3* similarly result in abnormalities of the jaw joint in Auriculo-Condylar Syndrome.<sup>60–64</sup> Absence of the jaw articulation is also seen in mice deleted for the Edn1 target gene *Dlx5* and *Dlx2*.<sup>65</sup> Somewhat differently, loss of *Foxc1* results in pronounced bony fusions of the upper and lower jaw, a condition known as syngnathia.<sup>66</sup> In addition to EDN1, BMP, and FGF signaling, WNT and HH signaling also play important roles in establishing joint domains.<sup>67–69</sup> In mice, *Wnt9a/Wnt14* is both necessary and sufficient to specify joint-forming domains in the limb,<sup>46,70</sup> and *Ihh* is required for limb and jaw joint development.<sup>67,71</sup> Conversely, HH activation has been found to inhibit joint development through repression of WNT signaling and *Fgf18* expression.<sup>69</sup> Going forward, it will be important to discern the extent to which these pathways establish early gene expression domains that prefigure joints versus more directly controlling chondrocyte and other cell fate at the joint (e.g., as discussed below for BMP signaling).

## CONTROLLING CHONDROCYTE IDENTITY AT THE JOINT

As the precartilaginous condensation forms, cells destined for both endochondral bone and the joint express a number of genes typical of mesenchymal progenitors, including *Sox9* and low levels of *Col2a1*.<sup>72</sup> In the anlagen of the endochondral bones, chondrocytes then upregulate cartilage matrix genes (e.g., *Col2a1*, *Matrilin1*, and *Aggrecan*) and stratify into proliferative, prehypertrophic, and hypertrophic zones.<sup>73</sup> Hypertrophic chondrocytes express *Col10a1* and low levels of genes in common with osteoblasts (e.g., *Runx2* and *Osterix*), mineralize, and undergo apoptosis or transdifferentiation into long-lived osteocytes.<sup>74–77</sup> In contrast, cells within the nascent interzone produce signaling molecules that help organize the joint and prevent further chondrocyte maturation. An ongoing debate is the extent to which joint chondrocytes arise from a separate lineage from those of endochondral bone. Lineage tracing studies based on *Col2a1* and *Dcx* transgenes suggest that endochondral and joint chondrocytes arise from a common early field of cells in mice,<sup>78,79</sup> and the observation that joint chondrocytes enter the endochondral program in certain zebrafish and mouse mutants would suggest that they share a common, albeit latent, potential with other chondrocytes in the condensation.<sup>4,28</sup> On the other hand, there are reports of migratory cells from outside the interzone contributing to articular chondrocytes and the meniscus in mouse,

which would imply an additional separate origin for at least some joint tissues.<sup>4,28,78,80</sup> Notwithstanding, it is clear that a number of signaling pathways and transcriptional repressors converge to potentially inhibit chondrocyte maturation at the developing joint surface.

### Thresholds of BMP and TGF $\beta$ Signaling for Chondrocyte Fate

Complex regulation of BMP signaling at and around the joint suggests a central role of this pathway in joint progenitor specification.<sup>81</sup> A variety of BMP ligands and antagonists are expressed in or around the developing interzone in mouse and zebrafish, including *Bmp2*, *Bmp4*, and *Bmp7*,<sup>82</sup> *Chordin*,<sup>12,27</sup> *Noggin*,<sup>83,84</sup> and *Gremlin2*,<sup>59</sup> and genetic evidence suggests that inhibiting *Bmp* signaling is critical for joint development. Transient misexpression of *Bmp4* in zebrafish, at a stage after its jaw patterning role, blocks formation of facial joints,<sup>28</sup> and loss of *Noggin* in the *Col2a1*-expressing lineage of mice disrupts interphalangeal joints.<sup>4</sup> On the other hand, loss of *Bmp2* and *Bmp4* in limb mesenchyme blocks formation of the elbow joint.<sup>85</sup> Of particular interest, the BMP ligands GDF5, GDF6, and GDF7 are prominently expressed within the joint interzone,<sup>26,27,86–89</sup> with *Gdf5*-Cre activity extensively labeling joint tissues in mice.<sup>8</sup> *Gdf5* was first identified as the gene mutated in the *brachypodism* mouse,<sup>90</sup> which displays limb and mild joint defects; mice doubly mutant for *Gdf5* and *Gdf6* show more extensive joint defects.<sup>87,88,90</sup> GDF5 activity is not, however, sufficient to induce joints, as GDF5 misexpression promotes rather than inhibits cartilage maturation.<sup>91,92</sup> One possibility is that high *Bmp* signaling further away from the joint promotes endochondral ossification, lower *Gdf* signaling near the joint promotes deeper articular chondrocyte fates, and a relative absence of *Bmp/Gdf* signaling at the joint surface preserves progenitors in an early chondrocyte/mesenchymal state (Figure 5). Members of the TGF $\beta$  signaling family are also critical for joint morphogenesis.<sup>93</sup> The *Tgfb2* receptor is expressed in the joint interzone, with its loss in mice resulting in a near complete absence of the interphalangeal joints due to ectopic hypertrophy of articular chondrocytes.<sup>94–96</sup> While differentiation of chondrocytes from human embryonic stem cells in the presence of BMP4 results in a hypertrophic identity, addition of TGF $\beta$  maintains chondrocytes in a nonhypertrophic state.<sup>97,98</sup> *Bmp/Gdf* and TGF $\beta$  signaling are thought to signal through a mixture of shared and distinct SMAD effectors.<sup>81</sup> Whereas competition for the common *Smad4* effector is predicted to result in cross-inhibitory actions of these pathways, the finding that mice lacking *Smad4* in the *Col2a1* lineage have only mild joint defects indicates that the balance of *Bmp/Gdf* and TGF $\beta$  pathways may be more important than the absolute levels of either.<sup>99</sup>

### Transcriptional Repression of Chondrocyte Maturation

Although lower levels of *Bmp* signaling are important for specifying joints, less is known about how such signaling is interpreted in the nucleus to repress the hypertrophic maturation of articular chondrocytes (Figure 6). *Cux1*<sup>100</sup> and the C-1-1 isoform of the Ets factor *Erg*<sup>101</sup> are both expressed at developing murine joints and can repress cartilage differentiation upon misexpression. Loss of *Erg* in *Gdf5*+ joint precursors did not, however, alter joint specification in mice, although it did lead to increased susceptibility to age- and injury-induced osteoarthritis of the knee.<sup>102</sup> Recent work in zebrafish has found roles for the Iroquois family of transcriptional repressors in joint specification. At the hyoid joint in

zebrafish, which functions in gill ventilation, prospective joint chondrocytes express SoxE factors but make only low levels of *Col2a1a* and Aggrecan. In animals lacking *irx7* and *irx5a*, hyoid joint chondrocytes inappropriately mature into high *Col2a1a*-expressing chondrocytes, which reflects direct repression of a *col2a1a* enhancer by Iroquois proteins.<sup>28</sup> In mammals, *Irx1* and *Irx2* are expressed in the developing interphalangeal joints,<sup>103</sup> and either mouse *IRX1* or zebrafish *Irx7* can repress the chondrogenic differentiation of a murine chondrogenic cell line.<sup>28</sup> In addition to Iroquois genes, the homolog of the Tricho-rhino-Phalangeal syndrome gene *TRPS1* is expressed at joints in zebrafish,<sup>104</sup> as well as in early chondrocytes of the murine growth plate.<sup>105,106</sup> Loss of *Trps1* results in precocious chondrocyte maturation in the condylar cartilage of the murine jaw joint,<sup>107</sup> and cone-shaped epiphyses, joint hypermobility, and osteoarthritis-like changes to articular cartilage in humans.<sup>108</sup> Interestingly, *IRX5* and *TRPS1* proteins form a complex during early neural crest development.<sup>109</sup> Given their similar expression and requirement in early chondrocytes, it will be interesting to test whether a similar protein–protein complex functions to inhibit cartilage maturation at joints. As *Trps1* and Iroquois genes are expressed in the perichondrium,<sup>28,110</sup> they may also have requirements outside of joints to inhibit chondrogenic differentiation.

### Epigenetic Fixation of Articular Cartilage Identity

It is becoming increasingly appreciated that covalent modifications of DNA and their associated histones can have long-term consequences on gene expression. A salient feature of joint cartilage is that it must be maintained as permanent hyaline cartilage for the life of the animal. An attractive model then is that epigenetic silencing of the chondrocyte hypertrophy program that helps lock articular cells into a permanent cartilage identity.<sup>111</sup> In cultured human chondrocytes, expression of *COL10A1* involves active DNA demethylation of CpG islands in its promoter.<sup>112</sup> In mice, loss of *Histone Deacetylase 4 (Hdac4)* results in accelerated hypertrophy and mineralization of cartilage, in part through increased histone acetylation and expression of *Runx2*.<sup>113</sup> Furthermore, treatment of synovium-derived cells from pig with TGF $\beta$ 1 induces chondrogenesis, with coexpression of *Hdac1* blocking the hypertrophy of these chondrocytes.<sup>114</sup> Similarly, the joint-promoting factor *NKX3.2* has been shown to function as a transcriptional repressor in complex with *HDAC1* and the common BMP/TGF $\beta$  effector, *SMAD4*, although the targets of *NKX3.2* remain unclear.<sup>115</sup> The transcriptional repressor *LRF* has also been shown to act together with *HDAC1* to repress chondrogenic differentiation in rodents.<sup>116</sup> These data suggest that DNA methylation and/or histone deacetylation of *Col10a1*, *Runx2*, and likely other genes associated with chondrocyte hypertrophy may be important for maintaining permanent cartilage at joints.

## GENERATION OF SYNOVIAL SPECIALIZATIONS

In addition to generating permanent articular cartilage, cells within the developing joint express a suite of genes involved in the creation of joint-specific structures (Figure 7). A prominent morphological event in synovial joints is the formation of a cavity between articular cartilage layers, which is accompanied by the loss of cell–cell contacts and production of molecular lubricants that promote joint function. In some joints, tissues with features in common with the articular cartilage layers can exist as menisci that project into

the cavity (e.g., in the knee joint) or as discs between the articular surfaces (e.g., in the mammalian jaw joint). Supportive ligaments also attach to specific sites in the joint through a transitional structure, the enthesis. Joints are clearly complex organs that vary greatly depending on their location in the body, with their development relying on integration of common joint-promoting programs with unique positional specifiers.

### Formation of Lubricated Cavities

Cavitation of the developing joint separates the two articular surfaces, yet the cellular mechanisms underlying this morphogenetic event remain unresolved. Different joints fail to cavitate in a number of mouse mutants, including the temporomandibular joint in *Trps1* mutants,<sup>107</sup> the digit joints in *Ihh* mutants,<sup>67</sup> and elbow and wrist joints in *Osr1; Osr2* double mutants.<sup>117</sup> In many of these cases, it remains unclear the extent to which lack of cavitation is secondary to earlier defects in joint specification. In one study, conditional deletion of the HH receptor *Smo* in *Sox9+* chondroprogenitors resulted in a failure of the disc to separate from the mandibular condyle but did not affect earlier joint specification.<sup>32</sup> Correlative evidence suggests that cavitation does not require extensive cell death within the developing interzone.<sup>118</sup> Instead, asymmetric synthesis of hyaluronan (HA), a major component of synovial fluid, may facilitate a decrease in cell–cell contacts that allows tissue separation.<sup>119</sup> In the mouse limb, conditional inactivation of *Has2*, a member of the HA synthase family, using a *Prrx1*-Cre line that is active in early limb mesenchyme, inhibits cavitation of digit joints and leads to an accumulation of mesenchymal cells in the presumptive joint space.<sup>120</sup> This effect is specific to *Has2*, as deletion of *Has1* and/or *Has3* does not affect cavitation.<sup>121</sup>

Synovial joints are also unique in producing lubricating fluid that reduces friction and protects joint integrity. A critical lubricating protein is the proteoglycan Lubricin, encoded by the *Prg4* gene, which complexes with Aggrecan and HA in the synovial fluid.<sup>122</sup> Within developing joints, *Prg4* expression appears only after *Gdf5* expression begins to subside, roughly correlating with initiation of cavitation.<sup>11</sup> In addition to expression in the most superficial cells of articular cartilage, *Prg4* expression can also be found in the synovium, meniscus, and ligaments of mammalian joints. Such shared expression may reflect combinatorial secretion of Lubricin from all cavity-lining tissues, as well as additional roles for Lubricin in the lubrication of nonjoint tissues such as ligaments.<sup>123</sup> Although dispensable for joint cavitation, Lubricin is required for the maintenance of many types of joints with osteoarthritic phenotypes seen in human camptodactyly arthropathy-coxa vara-pericarditis (CACP) syndrome patients,<sup>124</sup> mice lacking *Prg4*,<sup>11,125</sup> and zebrafish *prg4b* mutants.<sup>25</sup> The factors that induce *Prg4* expression during later joint development remain unclear, although a recent study suggests a potential role for mechanical forces<sup>126</sup> (see Box 1).

#### BOX 1

##### MECHANICAL FORCES IN JOINT DEVELOPMENTAL AND MAINTENANCE

Although genetics clearly plays an important role in joint development, mechanical forces also help to refine joint structure. For example, experimental immobilization of the developing avian limb revealed a requirement for movement in the cavitation but not

specification of joints.<sup>127,128</sup> Mechanical motion has also been shown to stimulate production of the major lubricant of synovial cavities, PRG4.<sup>126</sup> Imprecise joint architecture may also feed back through altered mechanosensation to locally modulate skeletal differentiation, in an attempt to correct joint structure. How cells in the joint sense mechanical forces and relay this information to modulate skeletal cell behavior remains largely a mystery. One clue is the identification of dominant mutations of *TRPV4* in congenital arthropathy in humans.<sup>129</sup> TRPV4 is a member of the Transient Receptor Potential class of gated calcium channels, and evidence suggests that it is a mechanosensitive channel that responds to compressive forces within the joint.<sup>130</sup> *Trpv4* is highly expressed by articular chondrocytes, and its loss in mice results in severe osteoarthritic changes in the knee joint.<sup>130,131</sup> Similarly, the PIEZO1 and PIEZO2 channels have been shown to be required for calcium signaling within cultured articular chondrocytes in response to directly applied mechanical forces.<sup>132</sup> What remains to be understood is how calcium signaling would result in changes in articular chondrocytes that maintain the health of the joint.

### Integrating Ligaments and Structural Components of the Joint

Menisci and discs are similar to the articular surface in having *Prg4*-expressing flattened cells overlying rounder chondrocyte-like cells. These structures arise within the *Gdf5*-expressing interzone, with their complex structures influenced by varied patterns of cavitation (e.g., discs are likely generated by two separate and parallel cavitation events). There are also specialized endochondral bones at joints that act as ligament attachment sites. These endochondral bones often form as secondary cartilage outgrowths from the bone surface (e.g., fossa of the mammalian jaw joint).<sup>71</sup> In zebrafish with reduced *nkx3.2* or *irx7* function, defects in articular cartilage are accompanied by loss of joint-associated endochondral bones at the jaw and hyoid joints, respectively, suggesting a high level of coordination of articular cartilage specification and joint-associated bone features.<sup>27,28</sup> The enthesis is a specialized transitional tissue between the endochondral bone and ligament at the joint. A common feature of ligament, enthesis, bone, and cartilage progenitors is expression of *Sox9*.<sup>133,134</sup> It is possible that there exists a common *Sox9*<sup>+</sup> progenitor pool within the early interzone, with local induction of lineage-specific factors (e.g., *Scleraxis* for ligaments) resulting in precise arrays of cartilage, bone, and ligament cells within the joint organ. It remains unclear whether interzone cells constitute a homogenous cell population that later diverges into distinct lineages, or whether interzone cells are heterogenous and lineage biased from initial stages.

### DEVELOPMENTAL PATHWAYS IN ADULT JOINT DISEASE

While genetic syndromes drastically affecting joint formation are rare, inherited susceptibility to joint degeneration (i.e., osteoarthritis) is common (Table 1). Osteoarthritis is thought to arise from accumulated “wear-and-tear” of joints. One possibility is that minor developmental defects lead to slightly abnormal joint architecture, which results in additive damage to the joint over time due to compromised function. Genetic programs that help specify articular at the expense of growth plate fates during development may also be

required to continuously maintain the articular chondrocyte phenotype through adulthood. These developmental pathways may also serve to maintain a progenitor pool in the postnatal joint, which in mammals has a limited ability to replace damaged joint tissue. Genetic changes that affect progenitor maintenance or function could thus impact joint homeostasis and predispose to osteoarthritis. Understanding roles for developmental genes in postnatal joint function and maintenance may therefore offer new insights into the cause and possible prevention of joint diseases.

### Do Subthreshold Developmental Defects Predispose to Osteoarthritis?

One theory is that minor alterations in patterning of joints during embryogenesis results in compromised postnatal joint structure and increased arthritis susceptibility.<sup>17</sup> Changes in the proliferation, survival, and differentiation of cartilage and bone precursors in the developing joint could all lead to alterations in normal joint architectures. For example, polymorphisms in *GDF5* are linked to early onset osteoarthritis in humans,<sup>142</sup> and mice lacking *Gdf5* have defects in joint formation, particularly in the digits.<sup>88</sup> As *Gdf5* expression peaks during initial joint formation and becomes much reduced by postnatal stages,<sup>11</sup> it appears likely that the osteoarthritis susceptibility associated with *GDF5* mutations is due to developmental defects at joints. Polymorphisms in other genes associated with the BMP, TGF $\beta$ , and WNT pathways have also been linked to osteoarthritis of the hip in humans, although causative effects of these mutations have not been shown in most cases. These include *BMP5*, the TGF $\beta$  inhibitor Asporin (*ASPN*), and the WNT inhibitor Frizzled-related protein (*FRZB*).<sup>16</sup> The roles of these pathways in early chondrocyte differentiation suggest that setting the proper balance of endochondral bone to permanent articular cartilage may be critical for long-term joint maintenance. Definitive proof that the developmental roles ascribed to these genes are what predispose to osteoarthritis would require selectively removing gene function only during development.

### Postnatal Maintenance of Permanent Articular Cartilage

Once established, permanent cartilage must be maintained at the joint surface through adulthood. The continued postnatal expression of many signaling components and transcription factors required for joint development suggest roles in joint maintenance, which in some cases is corroborated by osteoarthritic phenotypes upon postnatal gene deletion. Global expression profiling of bovine articular cartilage demonstrated decreased expression of TGF $\beta$  and BMP receptors with age,<sup>143</sup> and slow-cycling TGFBR2-expressing cells have been suggested to be resident joint stem cells involved in homeostasis (see Box 2). When *Bmpr1a* is conditionally deleted from the *Gdf5*<sup>+</sup> interzone in mice, joints of the ankle, knee, and digits display progressive loss of articular cartilage due to ectopic endochondral differentiation.<sup>9</sup> Stronger evidence implicates TGF $\beta$  signaling in adult maintenance of joints, as postnatal deletion of *Tgfb2* in murine articular cartilage, using *Col2a1*-CreER, results in ectopic chondrocyte hypertrophy at the joint surface and osteoarthritis of the knee.<sup>144</sup> *Trps1* and *Iroquois* genes, which are required to inhibit articular cartilage maturation during development, also display continued expression at joints, at least in zebrafish.<sup>28</sup> Similarly, *Wnt9a* expression is maintained in later joints<sup>72</sup> and the zone of Parathyroid hormone-related protein (PTHrP) expression subadjacent to articular cartilage is maintained in adult mice.<sup>145</sup> Deletion of PTHrP in *Gdf5*-Cre-expressing joint cells predisposes cartilage to

arthritis of the knee following destabilization of the medial meniscus in mice.<sup>146</sup> Furthermore, PTH analogs inhibit knee osteoarthritis progression in rats<sup>136</sup> and mice.<sup>147</sup> A failure of epigenetic maintenance of the permanent cartilage phenotype may also play a role in osteoarthritis progression. Genome-wide DNA methylation changes have been shown in hip and knee cartilage in human osteoarthritis patients<sup>137–139</sup> and have been linked to differential expression of 70 genes, including the osteoarthritis-associated genes *VIT*, *ROR2*, and *WLS*.<sup>140</sup> Altered activity of the Sirtuin family of histone deacetylases, in particular SIRT1, SIRT3, and SIRT6, has also been implicated in arthritis.<sup>19,148,149</sup> In particular, increased histone acetylation within articular cartilage has been linked to ectopic hypertrophy in the mouse knee, including increased expression of matrix metalloproteases (e.g., MMP-13) that degrade joint cartilage.<sup>150</sup>

## BOX 2

### STEM CELLS IN THE POSTNATAL JOINT

Researchers have sought to identify stem cells in the adult joint, both as potential endogenous therapeutic targets and as a source of transplantable cells for joint repair. Lineage tracing with tamoxifen-inducible *Prg4*-Cre revealed that *Prg4*<sup>+</sup> joint cells drive the growth of articular cartilage through adulthood in mice.<sup>3</sup> A subset of postnatal superficial joint cells also shows high colony-forming efficiency in culture.<sup>151</sup> In a disease context, chondrogenic progenitor cells have been isolated from late-stage human osteoarthritic cartilage and display multipotency, clonogenicity, and migratory potential *in vitro*.<sup>152</sup> BrdU labeling experiments have identified slow-cycling cells within the perichondrial zone of Ranvier at the periphery of the growth plate.<sup>141</sup> Additionally, TGFBR2-expressing cells in the “synovio-enthesal-articular cartilage niche,” which includes the zone of Ranvier and a subset of the perichondrium, synovium, and articular cartilage, have been shown to be slow cycling, thus representing a potential stem cell population in the adult joint.<sup>153</sup> When cultured *in vitro*, the synovium is expandable and chondrogenic,<sup>154,155</sup> and synovial cells respond both to acute injury<sup>156</sup> and joint disease<sup>124</sup> with increased proliferation, which may reflect a progenitor response toward repairing the joint. Postnatal lineage tracing of these potential stem cell sources is needed to clarify their role in joint homeostasis and repair. In the future, targeted activation of these potential stem cell populations could be a potential strategy for reversing joint damage.

While continuity of developmental programs may play a role in joint maintenance, there also appear to be factors with specific postnatal requirements at the joint, including Notch signaling,<sup>135,157,158</sup> PRG4,<sup>11,125</sup> and ERFF1.<sup>159</sup> The Notch pathway has been implicated in progenitor maintenance in a variety of contexts, including a role for inhibiting cartilage differentiation in mice<sup>160</sup> and zebrafish.<sup>161</sup> *Notch1* is expressed at postnatal joints in mice, and conditional deletion of the essential Notch effector RBP-Jk in either limb mesenchyme using *Prx1*-Cre or postnatal chondrocytes using *Col2a1*-CreER results in progressive joint cartilage degeneration and other osteoarthritic phenotypes.<sup>135,157,158</sup> The miR-140 is the major miRNA expressed in articular cartilage of murine joints,<sup>162</sup> and deletion of miR-140 predisposed mice to age-related osteoarthritis of the knee without affecting joint

specification.<sup>163</sup> Furthermore, miR-140 overexpression protects joints from injury-induced osteoarthritis.<sup>163</sup> One mechanism of miR-140 function is to inhibit the expression of *Adamts5*,<sup>163,164</sup> a metalloprotease that cleaves Aggrecan<sup>165</sup> and whose deletion mitigates osteoarthritis in mice.<sup>166</sup>

## CONCLUSION

The joint is a complex organ, composed of chondrocytes of different types and an array of associated connective tissues. Some of these connective tissues, in particular the synovium, may serve to not only encapsulate the joint cavity but also to act as a source of progenitors for new chondrocytes and joint tissues. A growing body of work is revealing how WNT, BMP/GDF, TGF $\beta$ , IHH, FGF, and other pathways are integrated in the developing joint interzone to control the expression of transcription factors and chromatin remodelers, which in turn locally specify cell fates. An important unanswered question is how members of the TGF $\beta$  superfamily—BMP, GDF, and TGF $\beta$ —are utilized to specify the different permanent cartilage zones at the articular surface versus the neighboring transient cartilage that converts into bone. Do the duration and/or relative levels of BMP versus TGF $\beta$  signaling drive different cartilage types? Or does signaling through distinct types of receptors and downstream effectors regulate different fates? A related question is how GDF and other signaling pathways regulate nonchondrocyte fates at joints. Chondrocytes, osteoblasts, and ligament cells all passage through a *Gdf5*+ interzone cell, with progenitors for each lineage also expressing *Sox9*. When do these lineages diverge from each other, and how are the major signaling pathways integrated differently to achieve these fates? An emerging theme is that there exists a continuum of cell fates throughout the joint, such as ligament cells that grade into osteoblasts in the enthesis, superficial chondrocytes that grade into synovial fibroblasts, and chondrocytes that grade into osteoblasts at the tidemark. Such graded fates may help link together the different components of the joint into a seamless organ.

A promising future research direction is to understand the extent to which the pathways that establish an articular cartilage fate function to maintain this fate, which in humans can be for over 100 years. An attractive but relatively unexplored possibility is that developmental pathways establish repressive DNA methylation and chromatin around genes associated with chondrocyte hypertrophy, thus locking articular chondrocytes into a permanent cartilage phenotype. Does the postnatal expression of transcriptional repressors such as Iroquois proteins and *Tips1* indicate a requirement for continued reinforcement of silencing of chondrocyte hypertrophy at the joint surface? And how would injury and decreased lubrication (e.g., due to *PRG4* loss) result in an override of epigenetic repression of chondrocyte hypertrophy? Relatedly, does failure to fully lock articular chondrocytes into a permanent cartilage identity during development predispose to later osteoarthritis? As such, new methods to assess chromatin status in joint tissue biopsies could result in the better diagnosis of susceptibility to arthritic changes, thus allowing for earlier detection and intervention before significant cartilage and bone erosion occurs.

While this review focuses on synovial joints, it will be interesting to understand the extent to which common pathways regulate cartilage and bone differentiation at other types of joints, such as intervertebral discs and sutures, as well as how their distinct utilization results in the

wonderful diversity of joint architectures throughout the body. Are these differences created by the different deployment of similar pathways, or by additional joint-specific genetic programs? In addition, it will be interesting to explore whether core sets of anti-differentiation pathways are used in other contexts. Do the same pathways that inhibit cartilage maturation at the articular surface also function to maintain undifferentiated cartilage progenitors in the perichondrium, or to extinguish cartilage gene expression as hypertrophic chondrocytes transdifferentiate into osteocytes in the mature growth plate? A related issue is whether the synovium functions as a modified perichondrium that can generate limited numbers of new articular chondrocytes to maintain the joint. If so, osteoarthritis may reflect less a complete inability to replace articular chondrocytes in adults, and more the inability of a slow homeostasis program to keep pace with repeated injuries to the joint during wear-and-tear. An important question then is whether genes required to specify articular cartilage during embryogenesis also play a more limited role in regenerating articular chondrocytes in adults. Alternatively, as recently described for adult bone homeostasis,<sup>167</sup> distinct progenitors may exist solely for postnatal joint homeostasis. In the future, a better understanding of how the different cell types of the joint are specified during development, then maintained and replaced as needed in the adult, will be critical for guiding new approaches toward both preventing and reversing osteoarthritis.

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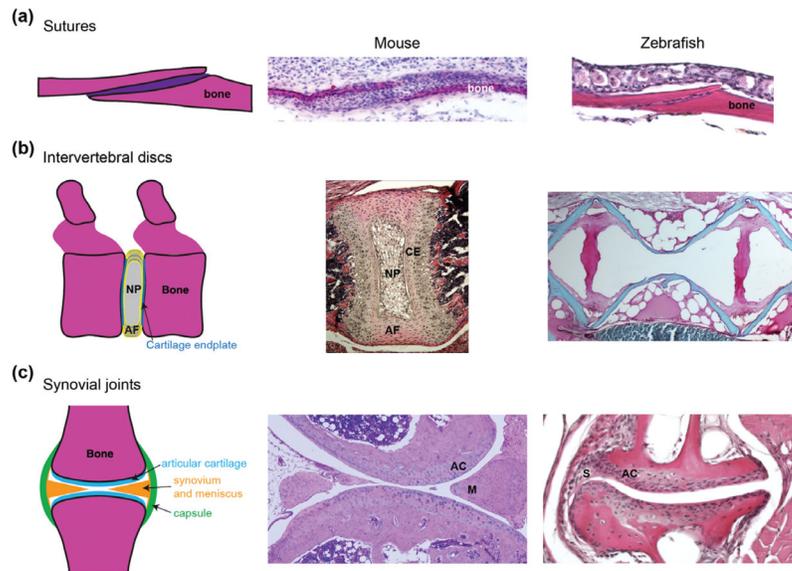
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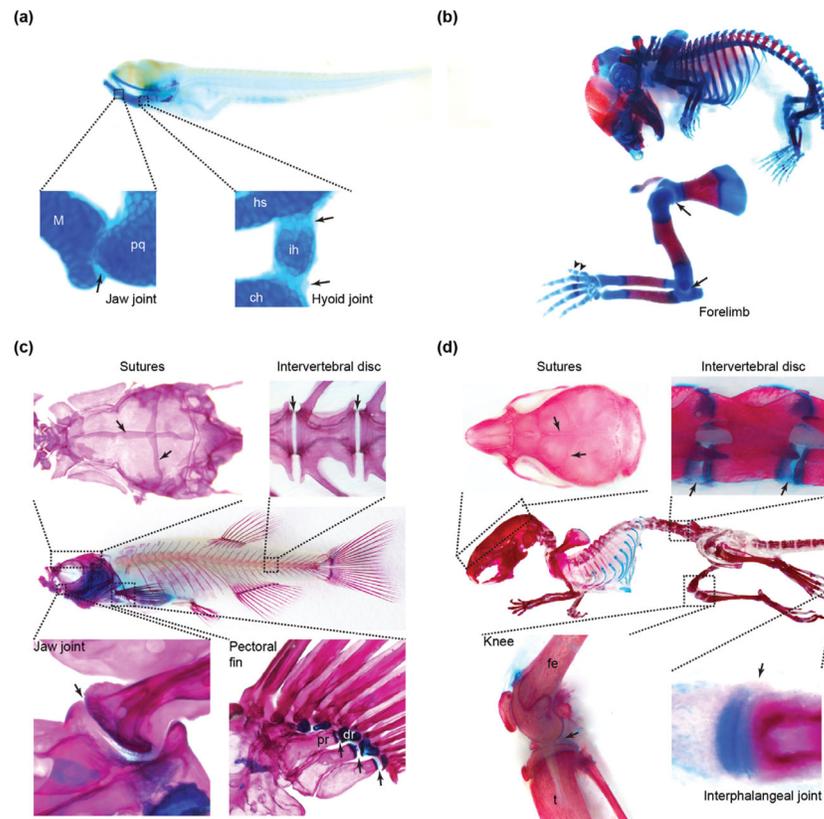
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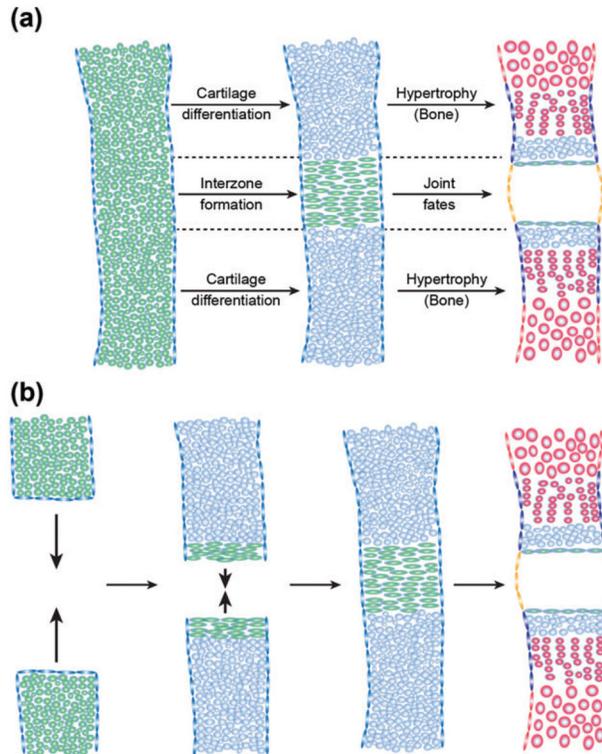
**FIGURE 1.**

Histological features of joints. (a) Sutures are a type of immovable articulation between bones, with the suture mesenchyme housing progenitors for bone growth and repair. Hematoxylin and eosin staining shows comparable coronal sutures of an E16.5 mouse embryo and young adult zebrafish. (b) The intervertebral discs of mouse and zebrafish have a very different structure. In mammals (shown here for postnatal Day 15 mouse), a cartilage endplate (CE) covers each vertebra, with the disc consisting of a ring of annulus fibrosus (AF) tissue and a core of nucleus pulposus (NP) tissue. In adult zebrafish, vertebral bones (red) are separated by layers of fat that appear white upon sectioning. (c) Synovial joints are freely moveable articulations characterized by fluid-filled cavities lined by articular hyaline cartilage. Some synovial joints, as shown in a section of the knee joint from an adult mouse, include additional specializations such as menisci (M). The adult zebrafish jaw joint has a clear articular cartilage (AC) layer and synovium (S) but no meniscus. Photos courtesy of Camilla Teng (A), Jennifer Zieba (B, mouse), and Denis Evseenko (C, mouse).



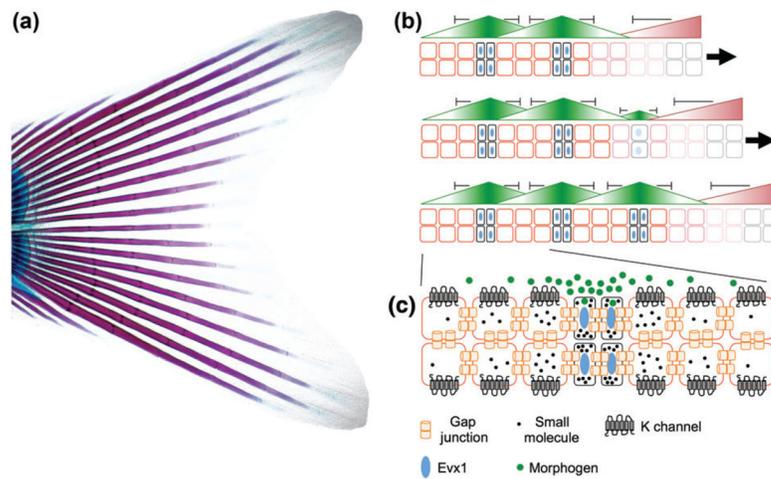
**FIGURE 2.**

Skeletal joints in zebrafish and mouse. (a) In zebrafish fry at 5 days postfertilization, well-studied cartilaginous joints (arrows) include the jaw joint between Meckel's and palatoquadrate cartilages and the bipartite hyoid joint between hyosymplectic, interhyal, and ceratohyal cartilages. (b) In a mouse embryo at E17.5, representative joints include the shoulder and elbow joints (arrows) and interphalangeal joints (arrowheads). As in young zebrafish, these joints are largely cartilaginous at this stage. (c) The adult zebrafish skeleton is largely composed of bone and contains many types of joints (arrows), including sutures in the skull, intervertebral joints, and synovial joints in the jaw and pectoral fin. (d) The mouse skeleton at postnatal Day 21 has similar joints to zebrafish, including sutures, intervertebral discs, and synovial joints in the knee and digits (i.e., interphalangeal). Skeletons were stained with alcian blue for cartilage and alizarin red for bone. ch, ceratohyal; dr, distal radial; fe, femur; hs, hyosymplectic; ih, interhyal; M, Meckel's; pq, palatoquadrate; pr, proximal radial; t, tibia.

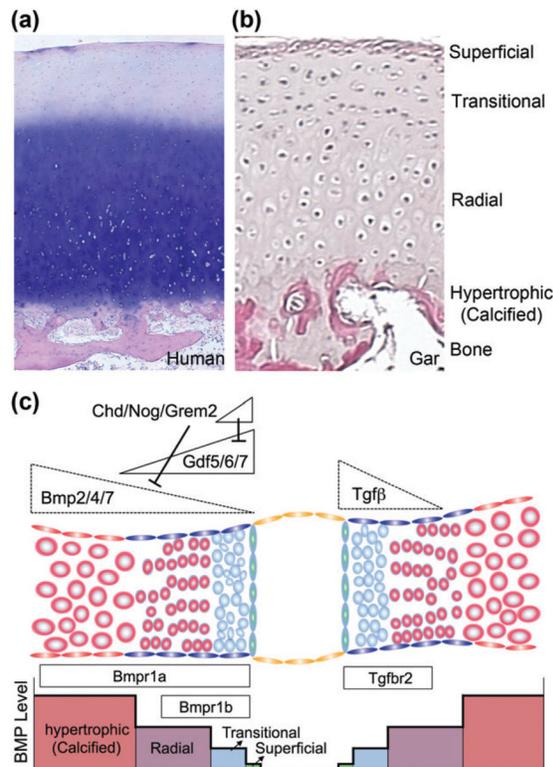


**FIGURE 3.**

Distinct modes of interzone formation. The joint interzone develops in the position of the presumptive joint and precedes articular cartilage differentiation and joint cavitation. The interzone can be generated from a single mesenchymal condensation (a, green) or through appositional growth of adjacent condensations (b). While cells outside the interzone undergo further cartilage differentiation (blue) and eventually hypertrophy and mineralize during endochondral bone development (red), cells within the interzone are maintained as immature chondrocytes at the articular surface (green flattened cells) and contribute to joint specializations such as the synovial membrane (orange). Also shown are chondrocyte progenitors within the perichondrium (blue flattened cells) and osteoblast progenitors within the periosteum (red flattened cells).

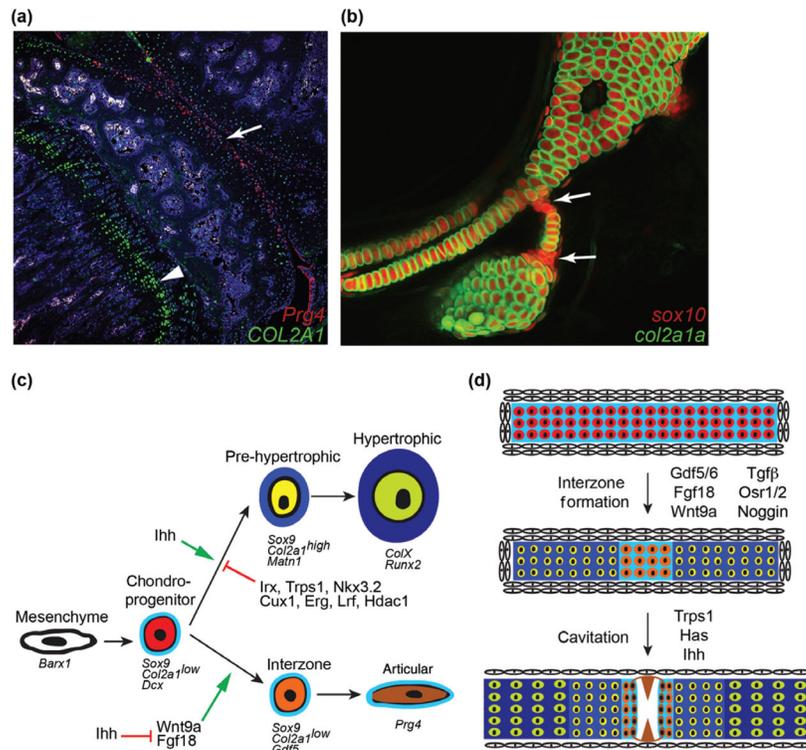
**FIGURE 4.**

Iterative segmentation of the bony rays of the zebrafish fin. (a) The tail fin from a 1-month-old zebrafish was stained with alizarin red and alcian blue to highlight bone and cartilage, respectively. In the bony rays, a series of joints form in a segmental pattern from the base of the fin to the tip (left to right in this image). (b) Modeling predicts that just two morphogens can generate the segmental pattern of joints in the fin rays. Bone cells are shown in red, joint cells in black with blue nuclei, and progenitors in gray. At forming joints, one morphogen (green) specifies joint fates while inhibiting neighboring cells from becoming joints. At the distal end, a growth factor (red) drives progenitor growth (right arrow) that lengthens the fin while inhibiting joint formation. Over time (top to bottom), new joints form where both morphogens fall below a critical threshold. (c) The gap junction protein Connexin43, the potassium channel *Kcnk5b*, and the transcription factor *Evx1* have all been shown to be required for correct joint spacing in the fin rays. One possibility is that secreted morphogens, as well as the transport of small molecules and electrical signaling between neighboring cells, combine to regulate joint spacing.



**FIGURE 5.**

Critical thresholds of Bmp and Tgf $\beta$  signaling in joint development. (a, b) Histological sections stained with hematoxylin and eosin show the different layers of joint cartilage in a human knee (16-year-old) and juvenile spotted gar jaw. (c) The levels, duration, and/or type of Bmp signaling help to specify the different types of chondrocytes at and around joints: hypertrophic, radial, transitional, and superficial. The Bmp ligands Gdf5/6/7 and antagonists Chordin (Chd), Noggin (Nog), and Gremlin2 (Grem2) are expressed in the developing interzone, and Bmp2/4/7 have been reported to be expressed either at a distance from the joint or at the joint itself. Variable diffusion of these ligands and antagonists may establish different levels of Bmp signaling, and signaling through Bmpr1a and Bmpr1b receptors could also influence joint fate. TGF $\beta$  signaling also has an important role to specify joint fates, with the Tgfr2 receptor enriched at developing joints.

**FIGURE 6.**

Chondrocyte fate decisions. (a) Section of the knee joint from an 8-week-old mouse shows that Col2a1 expression (green) is stronger in growth plate chondrocytes (arrowhead) compared to articular chondrocytes (arrow). Superficial joint chondrocytes are labeled by treatment of Prg4-CreER; Rosa26:memTomato/memGFP mice with Tamoxifen three weeks earlier (anti-GFP antibody staining detects Prg4-CreER-converted cells in red). (c) Compared to the complex mammalian knee joint, the hyoid joint of 6-day-old zebrafish (arrows) provides a simplified model for understanding the specification of joint chondrocyte fate. In this example, transient chondrocytes express both a *sox10:dsRed* transgene (red) and a *col2a1a:GFP* transgene (green). In contrast, joint chondrocytes express *sox10* but much lower levels of *col2a1a*, suggesting they are immature. (c) Cells within a mesenchymal condensation initially express *Barx1* and then go on to express *Sox9* (and in zebrafish also the related *SoxE* family member *sox10*), *Dcx*, and low levels of *Col2a1* (in particular an A splice isoform). In the growth plate, these cells mature into prehypertrophic chondrocytes that express high levels of *Col2a1* and *Matn1* and then hypertrophic chondrocytes that express *Col10a1*, *Runx2*, and other genes associated with mineralization. In contrast, interzone cells differentiate into articular chondrocytes that maintain low *Col2a1* and instead express *Gdf5* and later *Prg4*. Although *Wnt9a* and *Fgf18* promote a joint fate, *Ihh* signaling controls cartilage maturation. Specification of articular chondrocyte fate is promoted through inhibition of cartilage maturation by a number of transcription factors and chromatin remodelers, including Iroquois proteins (*Irx*), *Trps1*, *Nkx3.2*, *Cux1*, *Erg*, *Lrf*, and *Hdac1*. (d) Under the control of signaling factors including *Gdf5/6*, *Wnt9a*, *Fgf18*, *TGFβ* and *Noggin*, chondroprogenitor cells mature to form the different chondrocyte layers of the growth plate (darker blue) or remain relatively immature within the joint (orange cells in

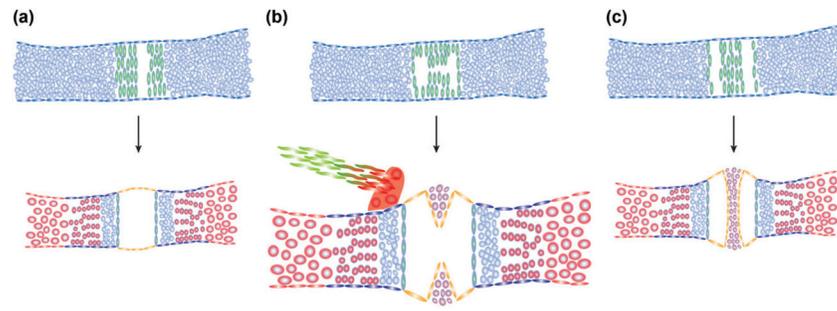
light blue matrix). Factors including *Trps1*, *Has*, and *Ihh* then drive interzone cells to cavitate as superficial zone articular chondrocytes (brown) begin to produce lubricating molecules such as *Prg4*.

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**FIGURE 7.**

Synovial specializations. Distinct patterns of cavitation of the interzone (green) can generate simple synovial joints (a) or joints with specialized structures such as menisci (b) and articular discs (c). The synovium (orange) shares many properties with the fibroblasts ensheathing the menisci and disc (purple). Bony processes (red) act as attachment points for ligaments (green), with their connection point, or enthesis, consisting of a transitional cell type.

**TABLE 1**  
Selected Human Joint Syndrome Genes and Animal Models of Joint Development and Disease

Gene Family	Genetic Models	Species	Human Syndrome	Joint Phenotype(s)	Selected References
<b>A. Joint cartilage specification</b>					
EDN1	<i>Edn1<sup>-/-</sup></i> <i>Ednra<sup>-/-</sup></i>	h, z	Auriculo-Condylar Syndrome (ARCND3) (OMIM: 615706)	Abnormal TMJ and mandibular condyle, loss of jaw joint	52-54
Nkx3.2	<i>nkx3.2</i> morpholino	z		Loss of jaw joint	27
Noggin	<i>Col2a1-Cre; Noggin<sup>fl/fl</sup></i>	h, m	Tarsal-carpal coalition syndrome (OMIM: 186570), proximal synphalangism (SYM1A) (OMIM: 185800)	Joint fusion (synphalangism), joint ankylosis, carpal and tarsal fusion, conductive deafness	4,83,84
FOXC1	<i>Foxc1<sup>-/-</sup></i>	m		Syngnathia (congenital adhesion of maxilla and mandible)	66
IHH	<i>Ihh<sup>-/-</sup></i>	m		Loss of limb joints, cavitation failure	67,71
GDF	<i>Gdf5<sup>-/-</sup>; Gdf6<sup>-/-</sup></i>	h, m	Proximal synphalangism (SYM1B) (OMIM: 615298)	Joint fusion at interphalangeal joints of fourth and fifth digit, digit joint fusion in mice	87,88,90
OSR1/OSR2	<i>Prrx1-Cre; Osr1<sup>fl/fl</sup>; Osr2<sup>-/-</sup></i>	m		Fusions at elbow joint, between the tibia and fibula, and carpal and tarsal joints; reduced <i>Pgs4</i> expression in articular cartilage	117
TGFβ	<i>Prrx1-Cre; Tgfb2<sup>fl/fl</sup></i>	m		Interphalangeal joint fusion	93,96
Connexin	<i>connexin43<sup>-/-</sup> Connexin40<sup>-/-</sup></i>	h, m, z	Oculodentodigital dysplasia (OMIM: 164200)	Loss of distal limb joints, shorter interjoint spacing, missing and fused phalanges	35,42,43
Knk5b	<i>knk5b</i> GOF mutation	z		Longer and irregular spacing of fin ray joints	37
Barx1	<i>barx1<sup>-/-</sup></i>	z		Ectopic joint in Meckel's cartilage of the jaw	33
BMP	<i>Prrx1-Cre; Bmp2<sup>CC</sup>; Bmp4<sup>CC</sup></i>	m		Elbow joint fusion	85
Irx	<i>irx7<sup>-/-</sup>; irx5a<sup>-/-</sup></i>	z		Hyoid joint fusion	28
<b>B. Cavitation</b>					
TRPS1	<i>Trps1<sup>-/-</sup></i>	h, m	Tricho-rhino-Phalangeal syndrome, type I (OMIM: 190350)	Temporomandibular joint cavitation defect, joint hypermobility	107,108
HAS	<i>Prrx1-Cre; Has2<sup>fl/fl</sup></i>	m		Carpal, metacarpophalangeal, interphalangeal, elbow and shoulder cavitation defects	120
<b>C. Joint Cartilage Maintenance</b>					
TRPV4	<i>TRPV4, Trpv4<sup>-/-</sup></i>	h, m	Familial digital arthropathy-brachydactyly (OMIM: 606835)	Defects of distal interphalangeal joints, early onset OA in fingers and toes, early onset OA of knee joints in mice	125,135
PRG4	<i>Prg4<sup>-/-</sup>; prg4b<sup>-/-</sup></i>	h, m, z	Camptodactyly arthropathy-coxa vara-pericarditis syndrome (OMIM: 208250)	Progressive failure of TMJ, ankle, knee, shoulder and elbow joints with age	11,25,30,124,125
PTHrP	<i>Gdf5-Cre; PTHrP<sup>fl/fl</sup></i>	m		Increased OA susceptibility following knee DMM surgery	133

Gene Family	Genetic Models	Species	Human Syndrome	Joint Phenotype(s)	Selected References
Sirtuin	<i>Sirt1</i> <sup>-/-</sup> , <i>Sirt6</i> <sup>+/+</sup>	m		Arthritis progression in knee joints	136,137
Notch	<i>Prrx1-Cre; RBP-Jk<sup>fl/fl</sup></i> , <i>Col2a1-CreER; RBP-Jk<sup>fl/fl</sup></i>	m		Cartilage degeneration in knee joints	138–140
microRNA	<i>miR-140</i> <sup>-/-</sup>	m		Increased age-related and surgery-induced OA susceptibility in knee joints	141

h, human; m, mouse; z, zebrafish.