[Frontiers in Bioscience 10, 576--581, January 1, 2005]

ANNEXINS – THEIR ROLE IN CARTILAGE MINERALIZATION

Thorsten Kirsch

Musculoskeletal Research Laboratories, Department of Orthopaedics, University of Maryland School of Medicine, 22 S. Greene Street, Baltimore, MD, USA

TABLE OF CONTENTS

1. ABSTRACT

Annexins II, V and VI are highly expressed by hypertrophic and terminally differentiated growth plate chondrocytes and by osteoblasts. Because of the localization of annexins in areas of cartilage and bone mineralization, we hypothesized that these annexins play a regulatory role in the mineralization process. In this article we review the function of annexins II, V and VI in physiological mineralization of skeletal tissues and in pathological mineralization of articular cartilage.

2. INTRODUCTION

Physiological mineralization occurring in growth plate cartilage, bone and teeth plays a major role during the development of these tissues. The mineralization process also allows these tissues to fulfill their proper functions during adulthood. Mineralization has to be restricted to certain areas. Mineralization in areas, where it normally should not occur, can have severe consequences. For example, mineral deposition in articular cartilage can lead to stimulation of matrix metalloproteinase (MMP) production and ultimately cartilage destruction (1). Mineralization of cardiovascular tissue leads to morbidity and mortality. Therefore, the mechanisms involved in regulating tissue mineralization are of great importance. Findings from our and other laboratories provided evidence that annexins, especially annexins II, V and VI are highly expressed in mineralizing growth plate chondrocytes and osteoblasts suggesting that these annexins are regulators of mineralization (2-4). This review will summarize major findings demonstrating the roles of annexins II, V and VI in physiological and pathological mineralization of skeletal and other tissues.

3. ANNEXINS II, V AND VI AND MATRIX VESICLE-MEDIATED MINERALIZATION OF GROWTH PLATE CARTILAGE

Annexins II, V and VI belong to a family of proteins possessing selective membrane-binding properties in the presence of calcium. These proteins are expressed in many different cell types and they were proposed to be involved in various functions, including anti-coagulation, anti-inflammation, exocytosis, membrane fusion, ion channels, and receptors for extracellular matrix proteins (5; 6). Annexins II, V and VI are highly expressed in the hypertrophic zone of growth plate cartilage and in bone in areas, where also mineralization occurs (2-4; 7). The annexins consist of a small N-terminal domain and a core domain. The core domain of annexin II and V contain 4 repeat units, while the core domain of annexin VI contains 8 repeat units (5; 6).

Physiological mineralization is restricted to bones, teeth and the hypertrophic zone of growth plate cartilage. This highly regulated process plays an important role during bone development and it also allows these tissues to fulfill their appropriate functions during adulthood. Ectopic or pathological mineralization can occur in all soft tissues but articular cartilage, skin, kidney, tendons, and cardiovascular tissue are particular prone to develop this pathology. Pathological mineralization of these tissues can lead to morbidity and mortality. Therefore an understanding of the regulatory mechanisms of mineralization is critical. Mineralization of growth plate cartilage, bone and teeth is initiated by matrix vesicles. Matrix vesicles are small membrane-enclosed particles, which are released of the plasma membrane of mineralization-competent cells, including chondrocytes, osteoblasts and odontoblasts. After these particles are released from the plasma membrane the first mineral phase forms inside these particles. Once the intravesicular mineral has reached a certain size it ruptures the vesicle membrane and grows out into the extracellular matrix (8). Since these vesicles are enclosed by a membrane, channel proteins are required to mediate the influx of mineral ions into these particles. Interestingly, annexins II, V and VI have been shown to exert calcium channel activities when inserted into artificial lipid bilayers or these proteins mediate Ca^{2+} influx into artificial liposomes (9-14). Based on these properties and the high expression of these annexins in mineralizing cartilage and bone areas we hypothesized that

annexins may form Ca^{2+} channels in matrix vesicles and mediate Ca^{2+} influx into these particles. We demonstrated that mineralization-competent matrix vesicles are only released by terminally differentiated growth plate chondrocytes undergoing mineralization, whereas non-mineralizing chondrocytes released vesicles, which cannot mediate the mineralization process (15). A major difference in the composition of these different vesicle fractions was that only matrix vesicles released from terminally differentiated, mineralization-competent growth plate chondrocytes contained high amounts of annexins II, V and VI, while vesicles released from non-mineralizing growth plate chondrocytes did not contain these annexins (15). We and others also demonstrated that annexins II, V and VI mediate Ca^{2+} influx into artificial liposomes (13; 14). A selective annexin Ca^{2+} channel blocker (K-201) was not only able to inhibit annexin II, V or VI-mediated Ca^{2+} influx into artificial liposomes, but this compound also inhibited Ca^{2+} influx and mineral formation by matrix vesicles, suggesting that annexin II, V and VI mediate Ca^{2+} influx into matrix vesicles and ultimately the formation of the first mineral phase within the vesicle lumen (12; 13). As mentioned above, vesicles isolated from non-mineralizing growth plate chondrocytes do not contain annexins II, V and VI and consequently these particles do not take up Ca^{2+} and initiate mineralization. However, the addition of annexin II, V or VI to these vesicles restored their ability to take up Ca^{2+} (13). Annexins are cytoplasmic proteins, which under certain conditions relocate to the plasma membrane (5). However, in order to form Ca^{2+} channels these annexins not only have to bind to the plasma membrane but they have to insert into the membrane. Previous findings have shown that annexins XII and V insert into the membrane and form channels under acidic conditions, whereas at neutral pH these annexins bind as multimers to the surface of the plasma membrane but do not form channels (16). Therefore, the formation of annexin channels is a highly regulated process, which can be regulated at different levels (gene expression, membrane binding and/or insertion). How do these annexins form Ca^{2+} channels under physiological conditions (e.g. in matrix vesicles)? Insertion of annexin V into lipid bilayers occurred at a pH of 5.0 (16). Interestingly, other findings have demonstrated that the pH at the cell periphery of late hypertrophic growth plate chondrocytes can be as low as 6.5 (17). Acidic conditions have also been detected in the synovial fluid of arthritic joints (18). Interestingly, arthritic cartilage expresses annexins (see ref 3). Furthermore, other studies have shown that the presence of certain lipids may facilitate annexin channel formation (19; 20). Therefore, a combination of factors, including pH and lipids, may facilitate annexin channel formation.

3.1. The Role of Collagen Binding of Annexin V in Matrix Vesicle-Mediated Mineralization

We and others have shown that annexin V binds to types II and X collagen (21; 22). Both collagens are present in the extracellular matrix of terminally differentiated mineralizing growth plate chondrocytes (23). The binding of types II and X collagen to annexin V has two significant consequences for matrix vesicle-mediated mineralization of growth plate chondrocytes: first, the binding anchors matrix vesicles to the extracellular matrix, and more importantly, this binding activates annexin V channel activities leading to an enhanced Ca^{2+} influx into liposomes (13). Matrix vesicles isolated from growth plate cartilage still contain types II and X collagen attached to their outer surface, whereas vesicles isolated from non-mineralizing growth plate chondrocytes do not contain annexin V and surface-attached types II and X collagen (15). Interestingly, selective removal of types II and X collagen from matrix vesicles isolated from mineralizing growth plate cartilage led to a drastic reduction of Ca^{2+} uptake by these vesicles and ultimately mineralization of these particles, whereas the addition of purified native type II or X collagen restored Ca^{2+} uptake by these 'collagen-depleted' matrix vesicles to levels similar to the ones of 'collagen-containing' matrix vesicles (24). Type II or X collagen after removal of their telopetide or globular regions was less effective in stimulating annexin V channel activities, whereas denatured type II or X collagen was not able to stimulate annexin V channel activities, suggesting that the non triple helical domains of type II and X collagen are involved in binding to annexin V and stimulating its Ca^{2+} channel activities (13; 24). Interestingly, previous findings have also demonstrated that bovine articular chondrocytes *in vitro* attach to type II collagen using annexin V. Attachment of these cells to type II collagen coated dishes was inhibited by antibodies specific for annexin V (25).

In summary, annexins II, V and VI play a major role in initiation of matrix vesicle-mediated mineralization of growth plate cartilage. These annexins form Ca^{2+} channels in matrix vesicles allowing the influx of Ca^{2+} into these particles. Types II and X collagen binding to annexin V activates its Ca^{2+} channel properties. These stimulated channel activities together with annexin II and VI Ca^{2+} channel activities allow rapid influx of Ca^{2+} and the formation of the first mineral phase inside matrix vesicles. Since the actual concentration of free Ca^{2+} is always low in matrix vesicles because most of Ca^{2+} is associated with the mineral phase, annexins continuously mediate Ca^{2+} influx into the vesicles (figure 1).

4. ANNEXINS REGULATE TERMINAL DIFFERENTIATION EVENTS AND RELEASE OF MINERALIZATION-COMPETENT MATRIX VESICLES FROM GROWTH PLATE CHONDROCYTES

Annexins not only form Ca^{2+} channels in matrix vesicles but they also form Ca^{2+} channels in terminally differentiated growth plate chondrocytes leading to the influx of Ca^{2+} into these cells (26; 27). Treatment of growth plate chondrocytes with retinoic acid, which stimulates terminal differentiation events (28), led to an increase of cytosolic Ca^{2+} concentration $[Ca^{2+}]_i$. This increase was inhibited by EDTA, the cytosolic Ca^{2+} chelator BAPTA, K-201, the specific annexin channel blocker, or antibodies specific for annexin II, V or VI (26). Blocking annexin-mediated increase of $[Ca^{2+}]_i$ resulted in decreased release of matrix vesicles, which contained alkaline phosphatase and annexins II, V and VI and which initiated

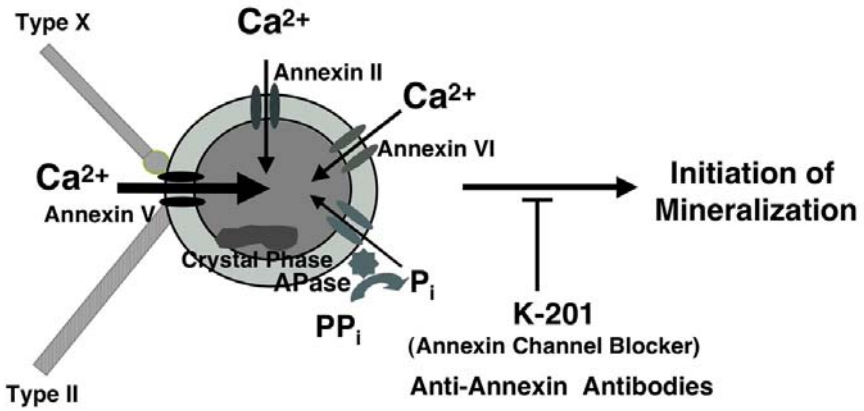

Figure 1. Schematic representation of matrix vesicle-mediated initiation of mineralization. Matrix vesicles initiate the mineralization process in growth plate cartilage, bones and teeth. Mineralization-competent matrix vesicles contain annexins II, V and VI, which form Ca^{2+} channels and enable the influx of Ca^{2+} into these particles. Types II and X collagen bind to annexin V and this binding stimulates annexin V Ca^{2+} channel activities. In addition, the vesicles contain alkaline phosphatase (APase) on their outer membrane surface. APase hydrolyzes pyrophosphate (PP_i) and other organic phospho-compounds and provides inorganic phosphate (P_i). P_i is transported into the vesicles through type III Na^+/P_i co-transporters. The influx of mineral ions (Ca^{2+} and P_i) leads to the formation of the first mineral phase inside the vesicle lumen and the initiation of mineralization. Matrix vesicle-mediated initiation of mineralization can be blocked by K-201, a specific annexin channel blocker, or antibodies specific for annexin II, V or VI.

mineralization (26). Furthermore, annexin-mediated Ca^{2+} influx into growth plate chondrocytes resulted in upregulation of mineralization-related genes, including alkaline phosphatase, osteocalcin and type I collagen. In addition, the expression of the transcription factor cbfa1 was also upregulated (27). Cbfa1 has been shown to play a major role in terminal chondrocyte differentiation. Overexpression of cbfa1 in non-hypertrophic chondrocytes resulted in an acceleration of endochondral ossification (29). Cbfa1 binds to the osteoblast-specific *cis*-acting element 2 (OSE2), which is found in the promoter regions of all the major terminal differentiation marker genes, including alkaline phosphatase, osteocalcin and type I collagen (30). Chelating intracellular Ca^{2+}, or inhibiting annexin Ca^{2+} channel activities using K-201 also resulted in a lower rate of apoptosis in terminal differentiated growth plate chondrocytes (27). Therefore, annexin-mediated Ca^{2+} influx into growth plate chondrocytes regulates a whole series of terminal differentiation events, including upregulation of mineralization and terminal differentiation marker genes, release of mineralization-competent matrix vesicles, mineralization of the extracellular matrix, and ultimately apoptosis (figure 2). Blocking annexin channel activities inhibits these terminal differentiation events, whereas overexpression of one of the annexins is sufficient to stimulate these events (27). As discussed above, chondrocytes seem also to attach to type II collagen using annexin V (25). Therefore, it is possible that binding of collagen via annexin V to the chondrocyte surface may affect terminal differentiation events by stimulating annexin V Ca^{2+} channel activities. Annexin V has been shown to mediate apoptosis in other cell types. B-lymphocytes lacking annexin V were resistant to Ca^{2+}-dependent apoptosis. Ca^{2+} has been demonstrated in many cell types to be required during apoptosis, and it is able to cause apoptosis by itself under conditions of Ca^{2+} overload. These studies have demonstrated that annexin V loss in B-lymphocytes also affects the release of cytochrome C from the mitochondria and consequently leads to the defects in apoptosis in these annexin V lacking B lymphocytes (31). Thus, it is possible that annexins not only affect differentiation events through their Ca^{2+} channel properties but also by affecting other cellular events.

5. ANNEXINS AND OSTEOARTHRITIS

Osteoarthritis, one of the most common diseases in elderly people, is characterized by a progressive destruction and loss of articular cartilage, and secondary inflammation processes. In contrast to growth plate chondrocytes, which have a limited life span and undergo a series of differentiation events eventually leading to apoptosis and replacement by bone, articular chondrocytes remain a stable phenotype throughout life (32). Findings from our and other laboratories demonstrated that normal articular cartilage does not express annexins II, V and VI. These annexins are, however, expressed by articular chondrocytes in osteoarthritic cartilage (2; 3; 33). Since annexins II, V and VI are only expressed in the hypertrophic and terminal differentiated zones of growth plate cartilage, these proteins can be considered markers of hypertrophy and terminal differentiation (2; 3). Interestingly, we and others found also other markers of chondrocyte hypertrophy and terminal differentiation, including alkaline phosphatase, osteocalcin, and osteopontin, are being expressed by articular chondrocytes in osteoarthritic cartilage (2; 34; 35). Mineral deposits, matrix vesicles associated with these mineral deposits, and apoptotic chondrocytes were also detected in these areas (2). These findings indicate that articular chondrocytes can lose their phenotype and undergo similar differentiation events as growth plate chondrocytes. Whereas these differentiation events are crucial for endochondral bone formation, they lead to destruction of articular cartilage. Whether similar factors, including retinoic acid, induce loss

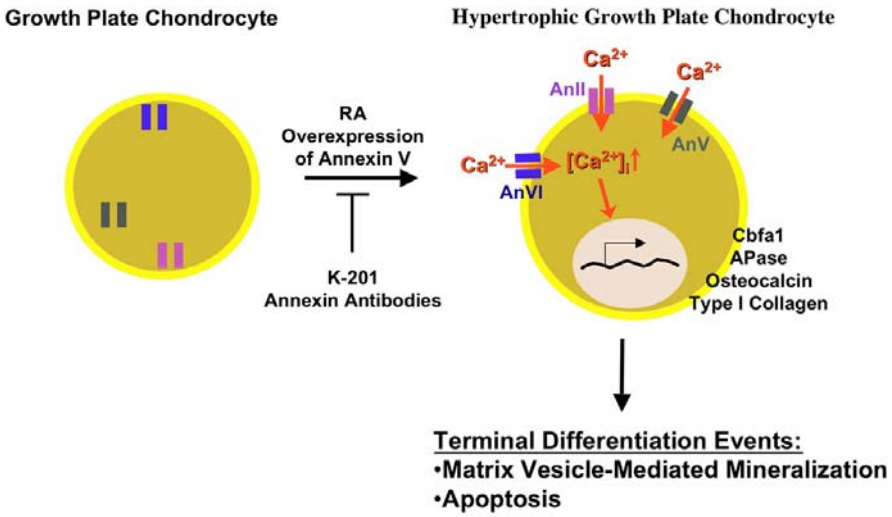

Figure 2. Schematic representation of the role of annexins II, V and VI in terminal differentiation events of growth plate chondrocytes. Treatment of retinoic acid (RA) or overexpression of one of the cartilage annexins (e.g. annexin V) results in channel formation of annexins II (AnII), V (AnV) and VI (AnVI) in the plasma membrane of growth plate chondrocytes and influx of Ca^{2+}. Annexin-mediated increase in cytosolic Ca^{2+} concentration $[Ca^{2+}]_i$ leads to upregulation of terminal differentiation and mineralization marker genes, including transcription factor cbfa1, alkaline phosphatase (APase), osteocalcin and type I collagen, mineralization, and eventually programmed cell death (apoptosis). K-201, a specific annexin Ca^{2+} channel blocker, or antibodies specific for annexin II, V or VI can inhibit these terminal differentiation events.

of articular chondrocyte phenotype and stimulation of hypertrophic and terminal differentiation events in these cells needs to be established. However, if annexins play a similar role in articular chondrocytes as in growth plate chondrocytes stimulating hypertrophic and terminal differentiation events, annexins may provide a novel therapeutic target to slow down the progression of osteoarthritis.

Furthermore, as discussed above, annexins play a major role in the initiation of mineralization by matrix vesicles. Excess mineralization has been implicated in several disease processes, including osteoarthritis and the calcification of cardiovascular tissues. Interestingly, vascular smooth muscle cells can undergo osteoblastic/chondrocytic differentiation as demonstrated by the expression of chondrocyte and osteoblast marker genes, including alkaline phosphatase, bone sialoprotein, osteocalcin, and cbfa1, and these cells release similar structures as matrix vesicles in calcified arteries (36; 37). If annexins are as central to pathogenic mineralization as they appear to be in mineralization of health tissue, they could be promising targets for a variety of therapies.

6. CONCLUSION

Annexins II, V and VI play a critical role in matrix vesicle-mediated mineralization of skeletal tissues. Annexin II, V and VI expression is upregulated in mineralization-competent tissue, and these annexins relocate to the plasma membrane followed by the release of annexin-containing matrix vesicles. These mineralization-competent matrix vesicles contain other components, including alkaline phosphatse, which are required for the initiation of mineralization. Once released from the plasma membrane, these annexins form Ca^{2+} channels in the vesicle membrane allowing the influx of Ca^{2+} required for the formation of the first mineral phase inside the vesicles. Interfering with annexin Ca^{2+} channel activities or removing surface-attached collagen, which binds to annexin V and stimulates its Ca^{2+} channel activities, resulted in inhibition of matrix vesicle-mediated mineralization. Furthermore, recent findings demonstrating that annexin II overexpression in osteoblasts resulted in increased alkaline phosphatase activity in lipid rafts and mineralization, suggest that annexins not only play a role in mediating Ca^{2+} influx into matrix vesicles but that these proteins may also play a major role in the formation and release of matrix vesicles from the plasma membrane of mineralization-competent cells (38). Finally, evidence was provided that annexins also form Ca^{2+} channels in hypertrophic growth plate chondrocytes leading to the influx of Ca^{2+} and stimulation of terminal differentiation events, including upregulation of terminal differentiation marker genes, release of mineralization-competent matrix vesicles, matrix mineralization and apoptosis. Furthermore, annexins may play a similar important role in pathological mineralization and therefore it is plausible that blocking the function of these proteins may provide a novel therapeutic target to inhibit excessive or pathological mineralization.

7. ACKNOWLEDGEMENT

Supported by grants from the National Institute for Arthritis and Musculoskeletal and Skin Diseases (AR 046245, AR 049074) and the Arthritis Foundation. We thank Drs. Jinping Xu and Wei Wang for their excellent technical assistance. We express our sincere gratitude to Drs. Noburu Kaneko (Dept. of Internal Medicine, Dokkyo University, Tochigi, Japan) and Toshizo Tanaka (Japan

Tobacco Inc., Central Pharmaceutical Research Institute, Osaka, Japan) for the generous gift of the compound K-201 (JTV519).

8. REFERENCES

1. McCarthy G. M., P. R. Westfall, I. Masuda, P. A. Christopherson, H. S. Cheung & P. G. Mitchell: Basic calcium phosphate crystals activate human osteoarthritic synovial fibroblasts and induce matrix metalloproteinase-13 (collagenase-3) in adult porcine articular chondrocytes. *Ann Rheum Dis* 60, 399-406 (2001)

2.Kirsch T., B. Swoboda & H.-D. Nah: Activation of annexin II and V expression, terminal differentiation, mineralization and apoptosis in human osteoarthritic cartilage. *Osteoarthritis and Cartilage* 8, 8294-8302 (2000)

3. Pfander D., B. Swoboda & T. Kirsch: Expression of early and late differentiation markers (proliferating cell nuclear antigen, syndecan-3, annexin VI, and alkaline phosphatase) by human osteoarthritic chondrocytes. *Am. J Pathol* 159, 1777-1783 (2001)

4.Suarez F., B. Rothhut, C. Comera, L. Touqui, F. R. Marie, C. Silve: Annexin-I, annexin-II, annexin-V, and annexin-VI by rat osteoblasts in primary culture – stimulation of annexin I by dexamethasone. *J Bone Miner Res* 8, 1201-1210 (1993)

5.Gerke V. & S. E. Moss: Annexins: from structure to function. *Physiol Rev* 82, 331-371 (2002)

6.Moss S. E. & R. O. Morgan: The Annexins. *Genome Biol* 5, (2004)

7. Mohiti J., A. M. Caswell & J. H. Walker: Calcium-induced relocation of annexins IV and V in the human osteosarcoma cell line MG-63. *Mol Membr Biol* 12, 321-329 (1995)

8. Anderson H. C.: Molecular biology of matrix vesicles. *Clin Orthop Rel Res* 314, 266-280 (1995)

9. Arispe N., E. Rojas, B. R. Genge, L. N. Y. Wu & R. E. Wuthier: Similarity in calcium channel activity of annexin v and matrix vesicles in planar lipid bilayers. *Biophys J* 711, 764-1775 (1996)

10. Benz J., A. Bergner, A. Hofmann, P. Demange, P. Gottig, S. Liemann, R. Huber & D. Voges: The structure of recombinant human annexin VI in crystals and membrane-bound. *J Mol Biol* 260, 638-643 (1996)

11. Burger A., R. Berendes, S. Liemann, J. Benz, A. Hofmann, P. Gottig, R. Huber, V. Gerke, C. Thiel, J. Romisch & K. Weber: The crystal structure and ion channel activity of human annexin II, a peripheral membrane protein. *J Mol Biol* 25, 7839-7847 (1996)

12. Kaneko N., R. Matsuda, M. Toda & K. Shimamoto: Inhibition of annexin V-dependent Ca^{2+} movement in large unilamellar vesicles by K201, a new 1,4-benzothiazepine derivative. *Biochim Biophys Acta-Biomemb* 1330, 1-7 (1997)

13. Kirsch T., G. Harrison, E. E. Golub & H.-D. Nah: The roles of annexins and types II and X collagen in matrix vesicle-mediated mineralization of growth plate cartilage. *J Biol Chem* 275, 35577-35583. (2000)

14. Matsuda R., N. Kaneko & Y. Horikawa: Presence and comparison of Ca^{2+} transport activity of annexins I, II, V, and VI in large unilamellar vesicles. *Biochem Biophys Res Commun* 237, 499-503 (1997)

15. Kirsch T., H. D. Nah, I. M. Shapiro & M. Pacifici: Regulated production of mineralization-competent matrix vesicles in hypertrophic chondrocytes. *J Cell Biol* 137, 1149-1160 (1997)

16. Isas J. M., J.-P. Cartailler, Y. Sokolov, D. R. Patel, R. Langen, H. Luecke, J. E. Hall & H. T. Haigler: Annexins V and XII insert into bilayers at mildly acidic pH and form ion channels. *Biochemistry* 39, 3015-3022 (2000)

17. Wu L. N. Y., M. G. Wuthier, B. R. Genge & R. E. Wuthier: In situ levels of intracellular Ca^{2+} and pH in avian growth plate cartilage. *Clin Orthop Rel Res* 335, 310-324 (1997)

18. Amraei M., Z. J. Jia, P. Reboul & I. R. Nabi: Acid-induced conformational changes in phosphoglucose isomerase result in its increased cell surface association and deposition on fibronectin fibrils. *J Biol Chem* 278, 38935-38941 (2003)

19. Kirsch T., H.-D. Nah, D. R. Demuth, G. Harrison, E. E. Golub, S. L. Adams & M. Pacifici: Annexin V-mediated calcium flux across membranes is dependent on the lipid composition. Implications for cartilage mineralization. *Biochemistry* 36, 3359-3367 (1997)

20. Isas J. M., D. R. Patel, C. Jao, S. Jayasinghe, J. P. Cartailler, H. T. Haigler & R. Langen: Global structural changes in annexin 12 - the roles of phospholipid, Ca^{2+}, and pH. *J Biol Chem* 278, 30227-30234 (2003)

21. Mollenhauer J., J. A. Bee, M. A. Lizarbe & K. von der Mark: Role of anchorin CII a 31000-molecular-weight membrane protein in interaction of chondrocytes with type II collagen. *J Cell Biol* 98, 1572-1578 (1984)

22. Kirsch T. & M. Pfaeffle: Selective binding of anchorin CII (annexin V) to type II and X collagen and to chondrocalcin (C-propeptide of type II collagen). *FEBS Lett* 310, 143-147 (1992)

23. Kirsch T. & K. von der Mark: Remodelling of collagen types I, II and X and calcification of human fetal cartilage. *Bone Miner* 18, 107-117 (1992)

24. Kirsch T. & R. E. Wuthier: Stimulation of calcification of growth plate cartilage matrix vesicles by binding to type II and X collagens. *J Biol Chem* 269, 11462-11469 (1994)

25. Reid D. L., M. B. Aydelotte & J. Mollenhauer: Cell attachment, collagen binding, and receptor analysis on bovine articular chondrocytes. *J Ortho Res* 18, 364-373 (2000)

26. Wang W. & T. Kirsch: Retinoic acid stimulates annexin-mediated growth plate chondrocyte mineralization. *J Cell Biol* 157, 1061-1069 (2002)

27. Wang W., J. Xu & T. Kirsch: Annexin-mediated Ca^{2+} influx regulates growth plate chondrocyte maturation and apoptosis. *J Biol Chem* 278, 3762-3769 (2003)

28. Iwamoto M., I. M. Shapiro, K. Yagami, A. L. Boskey, P. S. Leboy, S. L. Adams & M. Pacifici: Retinoic acid induces rapid mineralization and expression of mineralization-related genes in chondrocytes. *Exp Cell Res* 207, 413-420 (1993)

29. Takeda S., J. P. Bonnamy, M. J. Owen, P. Ducy & G. Karsenty: Continuous expression of Cbfa1 in nonhypertrophic chondrocytes uncovers its ability to induce hypertrophic chondrocyte differentiation and partially rescues Cbfa1-deficient mice. *Genes Dev* 15, 467-481 (2001)

30. Ducy P. & G. Karsenty: Two distinct osteoblast-specific *cis*-acting elements control expression of a mouse osteocalcin gene. *Mol Cell Biol* 15, 1858-6189 (1995)
31. Hawkins T. E., D. Das, B. Young & S. E. Moss: DT40 cells lacking the Ca^{2+}-binding protein annexin 5 are resistant to Ca^{2+}-dependent apoptosis. *Proc Natl Acad Sci U S A* 99, 8054-8059 (2002)
32. Kirsch T.: Osteoarthritis: a cellular defect? *Curr Opin Ortho* 14, 356-361 (2003)
33. Mollenhauer J., M. T. Mok, K. B. King, M. Gupta, S. Chubinskaya, H. Koepp & A. Cole: Expression of anchorin CII (cartilage annexin V) in human young, normal adult, and osteoarthritic cartilage. *J Histochem Cytochem* 47, 209-220 (1999)
34. Pullig O., G. Weseloh, S. Gauer & B. Swoboda: Osteopontin is expressed by adult human osteoarthritic chondrocytes: protein and mRNA analysis of normal and osteoarthritic cartilage. *Matrix Biol* 19, 245-255 (2000)
35. Pullig O., G. Weseloh, D. Ronneberger, S. Kakonen & B. Swoboda: Chondrocyte differentiation in human osteoarthritis: expression of osteocalcin in normal and osteoarthritic cartilage and bone. *Calcif Tissue Int* 67, 230-240 (2000)
36. Jono S., M. D. Mckee, C. E. Murry, A. Shioi, Y. Nishizawa, K. Mori, H. Morii & C. M. Giachelli: Phosphate regulation of vascular smooth muscle cell calcification. *Circ Res* 87, E10-E17 (2000)
37. Tyson K. L., J. L. Reynolds, R. Mcnair, Q. P. Zhang, P. L. Weissberg & C. M. Shanahan: Osteo/chondrocytic transcription factors and their target genes exhibit distinct patterns of expression in human arterial calcification. *Arteriosclerosis Thromb Vasc Biol* 23, 489-494 (2003)
Gillette J. M. & S. M. Nielsen-Preiss: The Role of Annexin 2 in osteoblastic mineralization. *J Cell Sci* 117, 441-449 (2004)

Key Words: Cartilage Biology, Calcium homeostasis, Differentiation, Growth Plate, Annexins, Matrix Vesicles, Mineralization, Osteoarthritis, Review

Send correspondence to: Thorsten Kirsch, Ph.D., Department of Orthopaedics, Musculoskeletal Research Laboratories, University of Maryland School of Medicine, 22 South Greene Street S11B, Baltimore, MD 21201, Tel: 410-706-2417, Fax: 410-706-0028, E-mail: tkirsch@umoa.umm.edu