HHS Public Access

Author manuscript

Biochim Biophys Acta. Author manuscript; available in PMC 2016 October 01.

Published in final edited form as:

Biochim Biophys Acta. 2015 October ; 1854(10 0 0): 1687–1693. doi:10.1016/j.bbapap.2015.03.015.

The Secretory Pathway Kinases

Anju Sreelatha[a], **Lisa N. Kinch**[b], and **Vincent S. Tagliabracci**[a,*]

[a]Department of Molecular Biology, University of Texas Southwestern Medical Center, Dallas, TX 75390, USA

[b]Department of Biochemistry, University of Texas Southwestern Medical Center, Dallas, TX 75390, USA

Abstract

Protein phosphorylation is a nearly universal post-translation modification involved in a plethora of cellular events. Even though phosphorylation of extracellular proteins had been observed, the identity of the kinases that phosphorylate secreted proteins remained a mystery until recently. Advances in genome sequencing and genetic studies have paved the way for the discovery of a new class of kinases that localize within the endoplasmic reticulum, Golgi apparatus and the extracellular space. These novel kinases phosphorylate proteins and proteoglycans in the secretory pathway and appear to regulate various extracellular processes. Mutations in these kinases cause human disease, thus underscoring the biological importance of phosphorylation within the secretory pathway.

Protein phosphorylation, catalyzed by protein kinases, is a post-translational modification that regulates various intracellular and extracellular processes. In the late nineteenth century, Olof Hammarsten detected phosphorous in the secreted milk protein, casein [1]. Despite its identification as the first phosphoprotein, the kinase responsible for phosphorylating casein had remained unknown until only recently [2]. In addition to casein, numerous secreted proteins, proteoglycans and peptide hormones are phosphorylated. However, the molecular identities of the kinases responsible for these modifications were unknown as well. As a consequence, research on extracellular protein phosphorylation has been largely undeveloped.

In the early days of protein kinase research, casein was used as an effective model substrate for the detection of protein kinase activity. In fact, casein was used by George Burnett and Eugene Kennedy in 1954 when they detected a novel enzyme activity from rat liver mitochondrial extracts that was capable of transferring the terminal phosphate of ATP to a proteinaceous substrate [3]. They called this enzyme “protein phosphokinase.” Today, we know of more than 540 such enzymes in humans that constitute the human “kinome” [4]. Following Burnett and Kennedy’s discovery, the enzymes responsible for the

*To whom correspondence should be addressed: vtagliabracci@ucsd.edu.

phosphorylation of casein in vitro were ultimately identified and named casein kinase 1 and casein kinase 2 (for a review of the casein kinases see [5]). However, these kinases localize in the nucleus and cytoplasm and would be unlikely to encounter casein within the secretory pathway. Therefore, to avoid confusion with the physiological casein kinase, which had been partially purified and biochemically characterized from highly enriched Golgi fractions, these enzymes were renamed protein kinase CK1 and protein kinase CK2 [6]. The molecular identity of the physiological casein kinase, however, remained elusive. Recently, a new family of atypical kinases, which localize within the secretory pathway and are secreted, was discovered (Figure 1) [7–10]. These enzymes are so different from canonical protein kinases that they were not included in the human kinome [4]. This family includes the Fam20C protein kinase, which is the physiological casein kinase; the xylose kinase, Fam20B; and the atypical cadherin kinase four-jointed (Fj). Furthermore, recent work has identified additional secretory pathway kinases, including the protein O-mannosyl kinase (Sgk196) and the secreted tyrosine kinase vertebrate lonesome kinase (VLK). Collectively, these enzymes comprise the secretory pathway kinases and they phosphorylate a diverse array of substrates that play important roles in many fundamental physiological processes. This review focuses on kinases within the secretory pathway and the extracellular space, with particular emphasis on their catalytic properties, structural features, and their importance in human physiology and disease.

Four jointed

Four-jointed (Fj) was first identified in *Drosophila* genetic studies in 1943 [11]. Mutations in Fj lead to the formation of only four of the five tarsal joints, hence giving rise to the name “four-jointed”. *Drosophila* Fj is a type II transmembrane protein, and its kinase domain faces the lumen of the secretory pathway [9]. Mutational studies implicated a role for Fj in the regulation of planar cell polarity in eye and wing development [12, 13]. One of the main regulators of planar cell polarity is the Fat signaling pathway [14]. Fat is a transmembrane protein receptor with multiple cadherin domains that form heterodimers with Dachsous (Ds), a transmembrane protein ligand with multiple cadherin domains [15, 16]. Early studies suggested that Fj regulates this interaction by acting as a ligand or modifier of the Fat-Ds signaling pathway. Golgi-retained Fj is still able to regulate cell polarity, suggesting it functions as a modifier rather than as an extracellular ligand [17]. In order to test if Fj modifies Fat or Ds, Ishikawa et al. coexpressed Ds and Fj to identify post-translational modifications [9]. These studies surprisingly revealed that Fj phosphorylates Ds and identified Fj as the first Golgi localized kinase. The carboxy terminus of Fj contains a Fam20C-like domain that is distantly related to canonical protein kinases [2]. Mutation of a conserved Fj ‘DNE’ motif, which corresponds to the canonical kinase ‘DFG’ activation segment motif, abolishes the phosphorylation activity on Fat and Ds without changing expression or localization of Fj [9]. Fj phosphorylates serine residues on the extracellular cadherin domains of Ds and decreases its binding affinity to Fat [9, 12, 18]. Conversely, phosphorylation of Fat by Fj increases its binding affinity to Ds [12]. These opposing forces dictate intercellular signaling to control polarization. The *Drosophila* studies shed light on a new class of kinases that function in the secretory pathway and phosphorylate extracellular substrates.

The mammalian orthologue of Fj, Fjx1, is less well studied, yet the regulation of planar cell polarity by Fjx1 may be conserved in mammals [19, 20]. However, Fjx1 does not appear to phosphorylate the vertebrate Fat1 cadherin in human cells [21]. Fjx1 is highly expressed in mouse brain, is a negative regulator of dendrite growth, and appears to be required for proper brain development [20, 22, 23]. Future studies are required to understand how Fjx1 is regulated and if there are additional substrates for Fjx1.

Fam20B

Studies in zebrafish identified Fam20B and xylosyltransferase-1 (Xylt-1) loss-of-function mutants as having decreased cartilage matrix and increased perichondral bone formation [24]. These genetic studies established that Fam20B and Xylt-1 operated in a common pathway to control proteoglycan biosynthesis. Subsequent biochemical work demonstrated that Fam20B phosphorylates the 2-OH on a xylose residue within the conserved tetrasaccharide linkage region of proteoglycans [10]. Xylose phosphorylation markedly stimulates the galactosyltransferase activity of galactosyltransferase II (GalT-II, B3GalT6), an enzyme that adds galactose to the growing tetrasaccharide linkage [25]. Furthermore, xylose phosphorylation appears to be necessary for EXTL2, a polymerase involved in the biosynthesis of heparin sulfate, to transfer a GlcNAc residue to the tetrasaccharide linkage region, which subsequently terminates chain elongation [26]. Thus, xylose phosphorylation may act as a quality control system for proteoglycan biosynthesis.

Genetic depletion of Fam20B in mice results in embryonic lethality at E13.5 and an analysis of the embryos revealed severe stunting, multisystem organ hypoplasia, and delayed development in the lung, eyes, liver, gastrointestinal tract and skeletal system [27]. Human *FAM20B* mutations have not been identified.

Fam20C

Fam20C is the physiological Golgi casein kinase and phosphorylates S-x-E/pS motifs on secreted proteins [2, 28]. The first 22 resides of Fam20C encode a predicted signal sequence that targets Fam20C to the secretory pathway where it encounters its substrates [29]. Removal of the signal sequence prevents Fam20C secretion and its ability to phosphorylate secreted proteins [2]. Fam20C has several unique characteristics for a protein kinase, including a preference for Mn^{2+} over Mg^{2+} as the metal ion cofactor and remarkable insensitivity to the broad-spectrum protein kinase inhibitor staurosporine [2, 28, 30]. Furthermore, Fam20C co-elutes with casein kinase activity when non-homogenized, non-pasteurized bovine milk is fractionated by ion exchange chromatography [2]. These biochemical characteristics, as well as the fact that Fam20C protein is enriched in crude Golgi casein kinase preparations [31], provide strong evidence that Fam20C is the physiological Golgi casein kinase.

In addition to casein, Fam20C phosphorylates several members of the small integrin binding ligand – N-linked glycoproteins (SIBLINGs) [2, 28]. The SIBLINGs consist of 5 identically oriented tandem genes that cluster within ~375Kbp on human chromosome 4. The genes encode osteopontin (OPN), dentin matrix protein-1 (DMP1), matrix extracellular phosphoglycoprotein (MEPE), bone sialoprotein (BSP), and dentin sialophosphoprotein

(DSPP). The SIBLINGs comprise a subfamily of the secretory calcium-binding phosphoproteins (SCPP) that regulate tooth and bone formation [32, 33]. Analysis of secreted phosphoproteomes suggest that Fam20C may not be restricted to the phosphorylation of proteins involved in calcium binding because more than two-thirds of human serum [34], plasma [35] and cerebrospinal fluid [36] phosphoproteins contain phosphate within the S-x-E/pS motif. These data, as well as the fact that Fam20C is found in organism that do not have mineralized tissues [8, 37], suggest that Fam20C may have many physiological functions beyond biomineralization.

VLK

While Fam20C phosphorylates serine residues of extracellular proteins, vertebrate lonesome kinase (VLK) phosphorylates tyrosine residues [38]. VLK was previously identified as a protein necessary for tissue morphogenesis and organogenesis during embryonic development [39–41]. VLK harbors an N-terminal signal sequence and a divergent C-terminal catalytic protein kinase-like domain that replaces the canonical kinase ‘DFG’ and ‘HRD’ motifs with alternate, yet functional residues. The VLK sequence belongs to a larger family of kinases (PKDCC) that are distantly related to a protein kinase family consisting of Fam69A/B/C and Deleted in Autism-1 (DIA1) and DIA1-Related. These proteins have not been functionally characterized; however, several of them have been genetically linked to neurological disorders and also are likely to function in the secretory pathway [42–46].

Bordoli et al. demonstrated that VLK is glycosylated and undergoes autophosphorylation in the secretory pathway [38]. Upon release from the cell, VLK phosphorylates proteins using extracellular ATP. Alternatively, VLK could use intracellular ATP sources to phosphorylate substrates in the Golgi. In contrast to Fjx1 and Fam20C, Mg^{2+} and Ca^{2+} rather than Mn^{2+} enhances VLK kinase activity. Furthermore, VLK phosphorylates a wide range of extracellular proteins with no recognizable consensus sequence. Matrix metalloproteinase 1 (MMP1), also known as collagenase I, is one such substrate of VLK. MMP1 is an enzyme that breaks down collagen in the extracellular matrix to promote cell migration and wound healing [47]. VLK phosphorylates MMP1 on tyrosine 360 of the hemopexin domain that dictates collagen binding; however, the functional consequences of MMP1 phosphorylation are currently unknown.

Several modes of VLK regulation have been proposed. VLK is primarily expressed in platelets and stimulation of platelets increases VLK secretion, suggesting a mode of regulation [38]. Additionally, deletion of the signal sequence or mutation of the catalytic residues result in a reduction of VLK secretion, suggesting that secretion may be regulated by kinase activity. Secretion of VLK could be regulated by autophosphorylation or by one of the substrates that are phosphorylated by VLK such as ERP29. ERP29 is an ER chaperone involved in proper folding and secretion of proteins involved in the secretory pathway [48]. VLK phosphorylates ERP29 on tyrosine 66 and this may, in turn, regulate VLK folding and secretion.

SGK196

SGK196 was identified in a haploid screen as a gene involved in the post-translational modification of α-dystroglycan [49]. Human mutations in *SGK196* lead to α-dystroglycan-related diseases such as congenital muscular dystrophy [49] (Table 1). Dystroglycan is composed of two subunits that serve as a link between the extracellular matrix and the cytoskeleton. The extracellular α-dystroglycan binds to ligands such as laminin, while the transmembrane β-dystroglycan indirectly binds to the actin cytoskeleton. α-dystroglycan is synthesized in the ER and glycosylated by the sequential actions of POMT1/2, POMGNT2/GTDC2, and B3GALNT2 to form a GalNAc-β3-GlcNAc-β4-mannose structure. The trisaccharide is then phosphorylated to allow glycan extension by enzymes including B4GALT1 and like-acetylglucosaminyltransferase (LARGE) [50–53]. SGK196, originally annotated as a pseudokinase [4], phosphorylates a sugar residue, *O*-mannose of N-acetlygalactosamine (GalNAc)- β3-N acetylglucosamine (GlcNAc)- β4-mannose, of α-dystroglycan [54]. Glycosylation and phosphorylation are required for α-dystroglycan to function as a receptor in intercellular signaling as well as in pathogen invasion. Defects in modifying enzymes lead to disorders collectively known as dystroglycanopathy characterized by brain and eye defects.

Secretory kinases in human disease

Raine Syndrome/FGF23-related hypophosphatemia

Loss of function mutations in Fam20C cause Raine Syndrome, a disorder first described in 1989 as a lethal osteosclerotic bone dysplasia [55] (Table 1). The original patient had microcephaly, a hypoplastic nose, exophthalmos, gum hyperplasia, cleft palate, and low set ears and died shortly after birth. In 2007, Simpson and colleagues identified mutations in the *FAM20C* gene as the cause of Raine Syndrome [56]. Raine Syndrome is extremely rare, with a prevalence estimated to be <1/1,000,000 [57]. Because of the limited number of cases (~35), a comprehensive phenotype is still not completely defined. Indeed, there appears to be significant clinical heterogeneity in patients, and not all *FAM20C* mutations are lethal. Notably, exome sequencing identified compound heterozygous mutations in Fam20C in two Norwegian brothers referred for fibroblast-growth factor-23 (FGF23)-related hypophosphatemia and dental defects. Likewise, FGF23-related hypophosphatemia was reported in a 61-year-old man with a homozygous Fam20C R408W mutation [58], and in a Brazilian family with a P496L mutation (Acevedo et al. BMC Med Genet; 2015).

Prior to these reports, Fam20C had been proposed to be a novel regulator of FGF23 because Fam20C null mice also develop hypophosphatemia due to an increase in circulating FGF23 [27, 59]. FGF23 is an endocrine hormone, secreted from osteoblasts and osteocytes, which primarily targets the kidney to regulate the re-absorption of phosphate and the production and catabolism of 1,25-dihydroxyvitamin D_3 [60]. Genetic and biochemical evidence have provided insight into the complex regulation of FGF23 processing and activity [61]. FGF23 is inactivated in the Golgi by proteolysis within a highly conserved subtilisin-like proprotein convertase (SPC) site, ^{176}RHTR↓SAE^{182} generating the N and C terminal fragments. Activating missense mutations in FGF23 (R176Q, R179W, and R179Q) cause autosomal dominant hypophosphatemic rickets (HR) [62]. These mutations substitute the Arg residues

within the SPC cleavage site and render the protein resistant to proteolysis [63]. Conversely, inactivating mutations in FGF23 cause HFTC, a hyperphosphatemic disorder characterized by ectopic calcifications in many tissues [64]. HFTC is also caused by loss-of-function mutations in polypeptide N-acetylgalactosaminyltransferase (GalNAc-T3), which prevent FGF23 O-glycosylation at Thr^{178} within the SPC cleavage site and render the hormone more susceptible to proteolysis [65, 66]. Fam20C phosphorylates FGF23 within the SPC site in vitro and in cells [67, 68]. This phosphorylation event inhibits O-glycosylation by GalNAc-T3, which then allows for proteolytic inactivation by furin (Figure 2). A missense mutation in Fam20C identified in the Norwegian brothers described above (T268M), has approximately 10% of the activity of the wildtype kinase (Cozza G…Pinna L et al. this issue). Furthermore, when expressed as a Flag-tagged fusion protein in the osteosarcoma cell line U2OS, Fam20C T268M is unable to completely inhibit O-glycosylation of secreted FGF23 [67]. These observations provide a plausible mechanism for the marked elevation of serum FGF23 protein observed in patients with *FAM20C* mutations and in *Fam20C* knockout mice [27, 59, 69].

In addition to direct interactions between Fam20C and FGF23, Fam20C likely also regulates FGF23 expression indirectly via the SIBLING and Fam20C substrate DMP1 (Figure 2). DMP1 is mutated in patients with an autosomal recessive form of hypophosphataemic rickets, and Dmp1 knockout mice have a similar phenotype to the Fam20C knockout mice [59, 70]. While the molecular mechanisms by which DMP1 regulates FGF23 levels are unknown, it appears that phosphorylation of DMP1 by Fam20C is necessary for the regulation of FGF23 expression. Consistent with this, overexpression of DMP1 in Fam20C knockout mice does not rescue the hypophosphatemia [71].

Amelogenesis Imperfecta with gingival fibromatosis syndrome (AIGFS) and Enamel Renal Syndrome

Using whole-exome sequencing, O'Sullivan and colleagues identified *FAM20A* mutations as a cause of AIGFS [72]. Amelogenesis Imperfecta (AI) refers to a group of disorders of improper enamel formation, and patients display genetic and clinical heterogeneity [73]. All of the enamel defects in patients with AIGFS, in addition to renal calcifications, are present in patients with Enamel-renal syndrome (ERS, OMIM 204690). Recently, Fam20A mutations were also identified in patients with ERS [27, 74, 75]. Consistently, Fam20A knockout mice have severe ectopic calcifications in the kidneys as well as pronounced enamel defects [27, 74]. Most human *FAM20A* mutations are inactivating and result in truncated versions of the protein. However, there have been missense mutations reported, and they are predicted to affect protein folding/stability (Table 1). For example, the G331D mutation would likely affect the local hydrophobic environment and produce an unstable/unfolded protein. Notably, Gly331 in Fam20A corresponds to Gly379 in Fam20C, a residue that is also mutated in Raine Syndrome (Table 1). Based on the high degree of sequence similarity between Fam20A and Fam20C, it is tempting to hypothesize that Fam20A is also a protein kinase that phosphorylates secreted proteins important for tooth enamel formation. Indeed, secretory ameloblasts secrete several phosphoproteins, some of which also cause AI when mutated [73]. However, structural-guided mutagenesis of Fam20C revealed several key catalytic residues that are important for phosphotransfer [76]. All of these residues

appear to be conserved in Fam20A except Glu306, which is a Gln in Fam20A (Q258). Glu306 is located in a loop preceding the αC helix and has a dual function: it coordinates the ion pair and the metal ion within the active site and is required for Fam20C kinase activity [76]. The presence of a Gln at this position in Fam20A suggests that Fam20A may not be an active kinase. In support of this, all of our attempts to demonstrate Fam20A kinase activity have yielded negative results. How Fam20A regulates enamel formation is currently unknown.

Structural Insights into Secretory Pathway Kinases

Crystal structures of the *Caenorhabditis elegans* Fam20C ortholog (ceFam20) were solved in the nucleotide free form, as well as in complex with ADP and Mn^{2+} [76]. As predicted by sequence analysis [2], the structure revealed an atypical protein kinase-like fold most similar to that of bacterial high persistence factor A (HipA), cell translocating kinase A (CtkA), and the slime mold actin-fragmin kinase (AFK). The ceFam20 structures highlight features specific to Fam20C, as well as provide insight into activities dictated by conserved canonical residues from the protein kinase-like core fold. Experimental data suggests that ceFam20 shares several characteristics with human Fam20C such as phosphorylation of S-x-E consensus motifs, preference of Mn^{2+} as a metal ion cofactor, and comparable kinetic constants. The crystal structure reveals important Fam20C residues that dictate these functions, including binding ATP, coordinating the metal ion cofactor, and catalyzing phosphate transfer. The structure also provides insight into Raine Syndrome and AIGFS disease causing mutations.

Fam20 from *C. elegans* exhibits a characteristic protein kinase-like core fold forming an active site at the interface of two lobes (Figure 3A). The fold is elaborated with several Fam20-specific features, including a unique N-terminal extension wrapped around the kinase core (residues 62–149), a Cys-rich subdomain inserted in the N-lobe (residues 243–288), a Fam20-specific loop (residues 194–213) preceding the αC helix common to all protein kinases, and a Fam20-specific loop (residues 311 – 323), that might dictate substrate specificity. Nucleotide binding is mediated in part by a glycine-containing loop (Gly-loop) located between the first and second N-lobe β-strands (Figure 3B). A conserved Gly-loop lysine (K178) binds the α- and β-phosphate of ADP. The α-phosphate is also coordinated by a conserved lysine (K192) that forms a salt bridge with a glutamic acid (E218) in the N-lobe αC helix. This ion pair is conserved in almost all protein kinases and provides a hallmark for the activation state [77]. The ion pair lysine also forms a unique salt bridge with a Fam20-specific insertion loop glutamic acid (E213) that coordinates Mn^{2+} together with the aspartic acid from the canonical kinase DFG motif (D387xG in Fam20). The catalytic aspartic acid from the kinase DRH (D366) motif forms a salt bridge with a neighboring arginine (R390). All of the same salt bridges are present in the absence of nucleotide, suggesting that the Fam20C apo kinase is competent for catalysis and need not be activated. The absence of an activation loop as well as the limited mobility of the N-lobe helix in Fam20C supports this conjecture [76].

The ceFam20 structure provides insight into disease-causing mutations found in Raine Syndrome and AIGFS. Three Fam20C mutations discovered in Raine patients (R408W,

P496L and T268M) occur in highly conserved positions and are predicted to effect substrate binding (Figure 3C). The R408W mutation (R314 in ceFam20C) replaces a positively charged side chain from a loop that abuts the catalytic segment. The corresponding loop from the related HipA structure (3fbr) helps form the peptide substrate binding site [78]. The nature of the Fam20C substrate motif (S-x-E/pS) suggests the selectivity might involve a basic residue, and the Raine Syndrome position R408 (ceFam20 R314) was proposed to play this role [76]. Mutation of this residue to a large hydrophobic side chain like tryptophan would likely impair the specificity of the binding pocket. The Raine Syndrome P496L mutation substitutes a helix-breaking proline (P405 in ceFam20C) that kinks αF adjacent to the proposed substrate specificity loop. The kink allows a Fam20 conserved aspartic acid (D399) from the following kinked turn to form a salt bridge with an arginine (R315) adjacent to the Raine Syndrome mutation position R408 (ceFam20 R314). This salt bridge is conserved in all Fam20 sequences and probably helps position the substrate specificity loop. Mutation of the helix-breaking proline to a larger leucine causes steric problems with the substrate specificity loop and may not allow the stabilizing salt bridge to form. The Raine Syndrome mutation T268M is in the nucleotide binding Gly-loop. The T175 side chain points towards R408 (ceFam20 R314) on the opposite side of the presumed substrate-binding groove. Mutation to a methionine might impair substrate binding. The AIGFS mutation G331D alters a small side chain from the Cys-rich cap. The same residue is mutated in Raine Syndrome G379E/R (G285 in ceFam20) (Table 1). Mutation of this residue to a larger hydrophobic side chain would destabilize the cap subdomain structure. In summary, the crystal structure of ceFam20 has revealed not only a unique architecture, but also has provided important mechanistic insight into several human disorders of biomineralization.

Conclusion

Phosphorylation is one of many post-translational modifications that increase the functional diversity of proteins inside and outside the cell. In the past decade, a new class of kinases that function in the secretory pathway and the extracellular space has been identified. Several of these kinases play critical roles in cell biology, and mutations lead to human disease. Understanding how these kinases are regulated and the identification of their substrates will help provide a better understanding of human physiology.

Acknowledgments

This work was supported by a K99/R00 Pathway to Independence Award from the National Institutes of Health (K99DK099254 to V.S.T).

References

1. Hammersten O. Zur Frage, ob das Casein ein einheitlicher Stoff sei. Zeitschrift für Physiologische Chemie. 1883; 7:227–273.
2. Tagliabracci VS, Engel JL, Wen J, Wiley SE, Worby CA, Kinch LN, Xiao J, Grishin NV, Dixon JE. Secreted kinase phosphorylates extracellular proteins that regulate biomineralization. Science. 2012; 336:1150–1153. [PubMed: 22582013]
3. Burnett G, Kennedy EP. The enzymatic phosphorylation of proteins. J Biol Chem. 1954; 211:969–980. [PubMed: 13221602]

4. Manning G, Whyte DB, Martinez R, Hunter T, Sudarsanam S. The protein kinase complement of the human genome. Science. 2002; 298:1912–1934. [PubMed: 12471243]
5. Venerando A, Ruzzene M, Pinna LA. Casein kinase: the triple meaning of a misnomer. Biochem J. 2014; 460:141–156. [PubMed: 24825444]
6. Allende JE, Allende CC. Protein kinases. 4. Protein kinase CK2: an enzyme with multiple substrates and a puzzling regulation. FASEB J. 1995; 9:313–323. [PubMed: 7896000]
7. Tagliabracci VS, Pinna LA, Dixon JE. Secreted protein kinases. Trends Biochem Sci. 2013; 38:121–130. [PubMed: 23276407]
8. Tagliabracci VS, Xiao J, Dixon JE. Phosphorylation of substrates destined for secretion by the Fam20 kinases. Biochemical Society transactions. 2013; 41:1061–1065. [PubMed: 23863179]
9. Ishikawa HO, Takeuchi H, Haltiwanger RS, Irvine KD. Four-jointed is a Golgi kinase that phosphorylates a subset of cadherin domains. Science. 2008; 321:401–404. [PubMed: 18635802]
10. Koike T, Izumikawa T, Tamura J, Kitagawa H. FAM20B is a kinase that phosphorylates xylose in the glycosaminoglycan-protein linkage region. Biochem J. 2009; 421:157–162. [PubMed: 19473117]
11. Waddington CH. The development of some ‘leg genes’ inDrosophila. Journal of Genetics. 1943; 45:29–43.
12. Simon MA, Xu A, Ishikawa HO, Irvine KD. Modulation of fat:dachsous binding by the cadherin domain kinase four-jointed. Curr Biol. 2010; 20:811–817. [PubMed: 20434335]
13. Zeidler MP, Perrimon N, Strutt DI. Multiple roles for four-jointed in planar polarity and limb patterning. Dev Biol. 2000; 228:181–196. [PubMed: 11112323]
14. Matakatsu H, Blair SS. Interactions between Fat and Dachsous and the regulation of planar cell polarity in the Drosophila wing. Development. 2004; 131:3785–3794. [PubMed: 15240556]
15. Clark HF, Brentrup D, Schneitz K, Bieber A, Goodman C, Noll M. Dachsous encodes a member of the cadherin superfamily that controls imaginal disc morphogenesis in Drosophila. Genes Dev. 1995; 9:1530–1542. [PubMed: 7601355]
16. Mahoney PA, Weber U, Onofrechuk P, Biessmann H, Bryant PJ, Goodman CS. The fat tumor suppressor gene in Drosophila encodes a novel member of the cadherin gene superfamily. Cell. 1991; 67:853–868. [PubMed: 1959133]
17. Strutt H, Mundy J, Hofstra K, Strutt D. Cleavage and secretion is not required for Four-jointed function in Drosophila patterning. Development. 2004; 131:881–890. [PubMed: 14757640]
18. Brittle AL, Repiso A, Casal J, Lawrence PA, Strutt D. Four-jointed modulates growth and planar polarity by reducing the affinity of dachsous for fat. Curr Biol. 2010; 20:803–810. [PubMed: 20434337]
19. Ashery-Padan R, Alvarez-Bolado G, Klamt B, Gessler M, Gruss P. Fjx1, the murine homologue of the Drosophila four-jointed gene, codes for a putative secreted protein expressed in restricted domains of the developing and adult brain. Mech Dev. 1999; 80:213–217. [PubMed: 10072791]
20. Rock R, Heinrich AC, Schumacher N, Gessler M. Fjx1: a notch-inducible secreted ligand with specific binding sites in developing mouse embryos and adult brain. Dev Dyn. 2005; 234:602–612. [PubMed: 16145673]
21. Sadeqzadeh E, de Bock CE, O’Donnell MR, Timofeeva A, Burns GF, Thorne RF. FAT1 cadherin is multiply phosphorylated on its ectodomain but phosphorylation is not catalysed by the four-jointed homologue. FEBS Lett. 2014; 588:3511–3517. [PubMed: 25150169]
22. Probst B, Rock R, Gessler M, Vortkamp A, Puschel AW. The rodent Four-jointed ortholog Fjx1 regulates dendrite extension. Dev Biol. 2007; 312:461–470. [PubMed: 18028897]
23. Saburi S, Hester I, Fischer E, Pontoglio M, Eremina V, Gessler M, Quaggin SE, Harrison R, Mount R, McNeill H. Loss of Fat4 disrupts PCP signaling and oriented cell division and leads to cystic kidney disease. Nat Genet. 2008; 40:1010–1015. [PubMed: 18604206]
24. Eames BF, Yan YL, Swartz ME, Levic DS, Knapik EW, Postlethwait JH, Kimmel CB. Mutations in fam20b and xylt1 reveal that cartilage matrix controls timing of endochondral ossification by inhibiting chondrocyte maturation. PLoS Genet. 2011; 7:e1002246. [PubMed: 21901110]
25. Wen J, Xiao J, Rahdar M, Choudhury BP, Cui J, Taylor GS, Esko JD, Dixon JE. Xylose phosphorylation functions as a molecular switch to regulate proteoglycan biosynthesis. Proc Natl Acad Sci U S A. 2014; 111:15723–15728. [PubMed: 25331875]

26. Nadanaka S, Zhou S, Kagiyama S, Shoji N, Sugahara K, Sugihara K, Asano M, Kitagawa H. EXTL2, a Member of the EXT Family of Tumor Suppressors, Controls Glycosaminoglycan Biosynthesis in a Xylose Kinase-dependent Manner. J Biol Chem. 2013; 288:9321–9333. [PubMed: 23395820]
27. Vogel P, Hansen GM, Read RW, Vance RB, Thiel M, Liu J, Wronski TJ, Smith DD, Jeter-Jones S, Brommage R. Amelogenesis imperfecta and other biomineralization defects in Fam20a and Fam20c null mice. Vet Pathol. 2012; 49:998–1017. [PubMed: 22732358]
28. Ishikawa HO, Xu A, Ogura E, Manning G, Irvine KD. The Raine syndrome protein FAM20C is a Golgi kinase that phosphorylates bio-mineralization proteins. PLoS One. 2012; 7:e42988. [PubMed: 22900076]
29. Bendtsen JD, Nielsen H, von Heijne G, Brunak S. Improved prediction of signal peptides: SignalP 3.0. J Mol Biol. 2004; 340:783–795. [PubMed: 15223320]
30. Lasa M, Marin O, Pinna LA. Rat liver Golgi apparatus contains a protein kinase similar to the casein kinase of lactating mammary gland. Eur J Biochem. 1997; 243:719–725. [PubMed: 9057837]
31. Lolli G, Cozza G, Mazzorana M, Tibaldi E, Cesaro L, Donella-Deana A, Meggio F, Venerando A, Franchin C, Sarno S, Battistutta R, Pinna LA. Inhibition of protein kinase CK2 by flavonoids and tyrphostins. A structural insight. Biochemistry. 2012; 51:6097–6107. [PubMed: 22794353]
32. Alvares K. The role of acidic phosphoproteins in biomineralization. Connect Tissue Res. 2014; 55:34–40. [PubMed: 24437603]
33. Kawasaki K. The SCPP gene family and the complexity of hard tissues in vertebrates. Cells Tissues Organs. 2011; 194:108–112. [PubMed: 21576905]
34. Zhou W, Ross MM, Tessitore A, Ornstein D, Vanmeter A, Liotta LA, Petricoin EF 3rd. An initial characterization of the serum phosphoproteome. J Proteome Res. 2009; 8:5523–5531. [PubMed: 19824718]
35. Carrascal M, Gay M, Ovelleiro D, Casas V, Gelpi E, Abian J. Characterization of the human plasma phosphoproteome using linear ion trap mass spectrometry and multiple search engines. J Proteome Res. 2010; 9:876–884. [PubMed: 19941383]
36. Bahl JM, Jensen SS, Larsen MR, Heegaard NH. Characterization of the human cerebrospinal fluid phosphoproteome by titanium dioxide affinity chromatography and mass spectrometry. Anal Chem. 2008; 80:6308–6316. [PubMed: 18702456]
37. Nalbant D, Youn H, Nalbant SI, Sharma S, Cobos E, Beale EG, Du Y, Williams SC. FAM20: an evolutionarily conserved family of secreted proteins expressed in hematopoietic cells. BMC Genomics. 2005; 6:11. [PubMed: 15676076]
38. Bordoli MR, Yum J, Breitkopf SB, Thon JN, Italiano JE Jr, Xiao J, Worby C, Wong SK, Lin G, Edenius M, Keller TL, Asara JM, Dixon JE, Yeo CY, Whitman M. A secreted tyrosine kinase acts in the extracellular environment. Cell. 2014; 158:1033–1044. [PubMed: 25171405]
39. Goncalves L, Filipe M, Marques S, Salgueiro AM, Becker JD, Belo JA. Identification and functional analysis of novel genes expressed in the Anterior Visceral Endoderm. Int J Dev Biol. 2011; 55:281–295. [PubMed: 21553379]
40. Imuta Y, Nishioka N, Kiyonari H, Sasaki H. Short limbs, cleft palate, and delayed formation of flat proliferative chondrocytes in mice with targeted disruption of a putative protein kinase gene, Pkdcc (AW548124). Dev Dyn. 2009; 238:210–222. [PubMed: 19097194]
41. Kinoshita M, Era T, Jakt LM, Nishikawa S. The novel protein kinase Vlk is essential for stromal function of mesenchymal cells. Development. 2009; 136:2069–2079. [PubMed: 19465597]
42. Dudkiewicz M, Lenart A, Pawlowski K. A novel predicted calcium-regulated kinase family implicated in neurological disorders. PLoS One. 2013; 8:e66427. [PubMed: 23840464]
43. Tennant-Eyles AJ, Moffitt H, Whitehouse CA, Roberts RG. Characterisation of the FAM69 family of cysteine-rich endoplasmic reticulum proteins. Biochem Biophys Res Commun. 2011; 406:471–477. [PubMed: 21334309]
44. Aziz A, Harrop SP, Bishop NE. DIA1R is an X-linked gene related to Deleted In Autism-1. PLoS One. 2011; 6:e14534. [PubMed: 21264219]
45. Aziz A, Harrop SP, Bishop NE. Characterization of the deleted in autism 1 protein family: implications for studying cognitive disorders. PLoS One. 2011; 6:e14547. [PubMed: 21283809]

46. Morrow EM, Yoo SY, Flavell SW, Kim TK, Lin Y, Hill RS, Mukaddes NM, Balkhy S, Gascon G, Hashmi A, Al-Saad S, Ware J, Joseph RM, Greenblatt R, Gleason D, Ertelt JA, Apse KA, Bodell A, Partlow JN, Barry B, Yao H, Markianos K, Ferland RJ, Greenberg ME, Walsh CA. Identifying autism loci and genes by tracing recent shared ancestry. Science. 2008; 321:218–223. [PubMed: 18621663]
47. Page-McCaw A, Ewald AJ, Werb Z. Matrix metalloproteinases and the regulation of tissue remodelling. Nat Rev Mol Cell Biol. 2007; 8:221–233. [PubMed: 17318226]
48. Hsu PP, Kang SA, Rameseder J, Zhang Y, Ottina KA, Lim D, Peterson TR, Choi Y, Gray NS, Yaffe MB, Marto JA, Sabatini DM. The mTOR-regulated phosphoproteome reveals a mechanism of mTORC1-mediated inhibition of growth factor signaling. Science. 2011; 332:1317–1322. [PubMed: 21659604]
49. Jae LT, Raaben M, Riemersma M, van Beusekom E, Blomen VA, Velds A, Kerkhoven RM, Carette JE, Topaloglu H, Meinecke P, Wessels MW, Lefeber DJ, Whelan SP, van Bokhoven H, Brummelkamp TR. Deciphering the glycosylome of dystroglycanopathies using haploid screens for lassa virus entry. Science. 2013; 340:479–483. [PubMed: 23519211]
50. Inamori K, Yoshida-Moriguchi T, Hara Y, Anderson ME, Yu L, Campbell KP. Dystroglycan function requires xylosyl- and glucuronyltransferase activities of LARGE. Science. 2012; 335:93–96. [PubMed: 22223806]
51. Yoshida-Moriguchi T, Yu L, Stalnaker SH, Davis S, Kunz S, Madson M, Oldstone MB, Schachter H, Wells L, Campbell KP. O-mannosyl phosphorylation of alpha-dystroglycan is required for laminin binding. Science. 2010; 327:88–92. [PubMed: 20044576]
52. Willer T, Inamori K, Venzke D, Harvey C, Morgensen G, Hara Y, Beltran Valero de Bernabe D, Yu L, Wright KM, Campbell KP. The glucuronyltransferase B4GAT1 is required for initiation of LARGE-mediated alpha-dystroglycan functional glycosylation. eLife. 2014; 3
53. Praissman JL, Live DH, Wang S, Ramiah A, Chinoy ZS, Boons GJ, Moremen KW, Wells L. B4GAT1 is the priming enzyme for the LARGE-dependent functional glycosylation of alpha-dystroglycan. eLife. 2014; 3
54. Yoshida-Moriguchi T, Willer T, Anderson ME, Venzke D, Whyte T, Muntoni F, Lee H, Nelson SF, Yu L, Campbell KP. SGK196 is a glycosylation-specific O-mannose kinase required for dystroglycan function. Science. 2013; 341:896–899. [PubMed: 23929950]
55. Raine J, Winter RM, Davey A, Tucker SM. Unknown syndrome: microcephaly, hypoplastic nose, exophthalmos, gum hyperplasia, cleft palate, low set ears, and osteosclerosis. J Med Genet. 1989; 26:786–788. [PubMed: 2614802]
56. Simpson MA, Hsu R, Keir LS, Hao J, Sivapalan G, Ernst LM, Zackai EH, Al-Gazali LI, Hulskamp G, Kingston HM, Prescott TE, Ion A, Patton MA, Murday V, George A, Crosby AH. Mutations in FAM20C are associated with lethal osteosclerotic bone dysplasia (Raine syndrome), highlighting a crucial molecule in bone development. Am J Hum Genet. 2007; 81:906–912. [PubMed: 17924334]
57. Faundes V, Castillo-Taucher S, Gonzalez-Hormazabal P, Chandler K, Crosby A, Chioza B. Raine syndrome: an overview. European journal of medical genetics. 2014; 57:536–542. [PubMed: 25019372]
58. Takeyari S, Yamamoto T, Kinoshita Y, Fukumoto S, Glorieux FH, Michigami T, Hasegawa K, Kitaoka T, Kubota T, Imanishi Y, Shimotsuji T, Ozono K. Hypophosphatemic osteomalacia and bone sclerosis caused by a novel homozygous mutation of the FAM20C gene in an elderly man with a mild variant of Raine syndrome. Bone. 2014; 67:56–62. [PubMed: 24982027]
59. Wang X, Wang S, Li C, Gao T, Liu Y, Rangiani A, Sun Y, Hao J, George A, Lu Y, Groppe J, Yuan B, Feng JQ, Qin C. Inactivation of a novel FGF23 regulator, FAM20C, leads to hypophosphatemic rickets in mice. PLoS Genet. 2012; 8:e1002708. [PubMed: 22615579]
60. White KE, Hum JM, Econs MJ. Hypophosphatemic rickets: revealing novel control points for phosphate homeostasis. Current osteoporosis reports. 2014; 12:252–262. [PubMed: 24980542]
61. Wolf M, White KE. Coupling fibroblast growth factor 23 production and cleavage: iron deficiency, rickets, and kidney disease. Current opinion in nephrology and hypertension. 2014; 23:411–419. [PubMed: 24867675]

62. Autosomal dominant hypophosphataemic rickets is associated with mutations in FGF23. Nat Genet. 2000; 26:345–348. [PubMed: 11062477]

63. White KE, Carn G, Lorenz-Depiereux B, Benet-Pages A, Strom TM, Econs MJ. Autosomal-dominant hypophosphatemic rickets (ADHR) mutations stabilize FGF-23. Kidney Int. 2001; 60:2079–2086. [PubMed: 11737582]

64. Benet-Pages A, Orlik P, Strom TM, Lorenz-Depiereux B. An FGF23 missense mutation causes familial tumoral calcinosis with hyperphosphatemia. Hum Mol Genet. 2005; 14:385–390. [PubMed: 15590700]

65. Topaz O, Shurman DL, Bergman R, Indelman M, Ratajczak P, Mizrachi M, Khamaysi Z, Behar D, Petronius D, Friedman V, Zelikovic I, Raimer S, Metzker A, Richard G, Sprecher E. Mutations in GALNT3, encoding a protein involved in O-linked glycosylation, cause familial tumoral calcinosis. Nat Genet. 2004; 36:579–581. [PubMed: 15133511]

66. Kato K, Jeanneau C, Tarp MA, Benet-Pages A, Lorenz-Depiereux B, Bennett EP, Mandel U, Strom TM, Clausen H. Polypeptide GalNAc-transferase T3 and familial tumoral calcinosis. Secretion of fibroblast growth factor 23 requires O-glycosylation. J Biol Chem. 2006; 281:18370–18377. [PubMed: 16638743]

67. Tagliabracci VS, Engel JL, Wiley SE, Xiao J, Gonzalez DJ, Nidumanda Appaiah H, Koller A, Nizet V, White KE, Dixon JE. Dynamic regulation of FGF23 by Fam20C phosphorylation, GalNAc-T3 glycosylation, and furin proteolysis. Proc Natl Acad Sci U S A. 2014; 111:5520–5525. [PubMed: 24706917]

68. Lindberg I, Pang HW, Stains JP, Clark D, Yang AJ, Bonewald L, Li KZ. FGF23 is endogenously phosphorylated in bone cells. J Bone Miner Res. 2014

69. Rafaelsen SH, Raeder H, Fagerheim AK, Knappskog P, Carpenter TO, Johansson S, Bjerknes R. Exome sequencing reveals FAM20c mutations associated with FGF23-related hypophosphatemia, dental anomalies and ectopic calcification. J Bone Miner Res. 2013

70. Feng JQ, Ward LM, Liu S, Lu Y, Xie Y, Yuan B, Yu X, Rauch F, Davis SI, Zhang S, Rios H, Drezner MK, Quarles LD, Bonewald LF, White KE. Loss of DMP1 causes rickets and osteomalacia and identifies a role for osteocytes in mineral metabolism. Nat Genet. 2006; 38:1310–1315. [PubMed: 17033621]

71. Wang X, Wang J, Yuan B, Lu Y, Feng JQ, Qin C. Overexpression of Dmp1 fails to rescue the bone and dentin defects in Fam20C knockout mice. Connective tissue research. 2014; 55:299–303. [PubMed: 24874551]

72. O'Sullivan J, Bitu CC, Daly SB, Urquhart JE, Barron MJ, Bhaskar SS, Martelli-Junior H, dos Santos Neto PE, Mansilla MA, Murray JC, Coletta RD, Black GC, Dixon MJ. Whole-Exome sequencing identifies FAM20A mutations as a cause of amelogenesis imperfecta and gingival hyperplasia syndrome. Am J Hum Genet. 2011; 88:616–620. [PubMed: 21549343]

73. Stephanopoulos G, Garefalaki ME, Lyroudia K. Genes and related proteins involved in amelogenesis imperfecta. J Dent Res. 2005; 84:1117–1126. [PubMed: 16304440]

74. Wang SK, Aref P, Hu Y, Milkovich RN, Simmer JP, El-Khateeb M, Daggag H, Baqain ZH, Hu JC. FAM20A mutations can cause enamel-renal syndrome (ERS). PLoS Genet. 2013; 9:e1003302. [PubMed: 23468644]

75. Jaureguiberry G, De la Dure-Molla M, Parry D, Quentric M, Himmerkus N, Koike T, Poulter J, Klootwijk E, Robinette SL, Howie AJ, Patel V, Figueres ML, Stanescu HC, Issler N, Nicholson JK, Bockenhauer D, Laing C, Walsh SB, McCredie DA, Povey S, Asselin A, Picard A, Coulomb A, Medlar AJ, Bailleul-Forestier I, Verloes A, Le Caignec C, Roussey G, Guiol J, Isidor B, Logan C, Shore R, Johnson C, Inglehearn C, Al-Bahlani S, Schmittbuhl M, Clauss F, Huckert M, Laugel V, Ginglinger E, Pajarola S, Sparta G, Bartholdi D, Rauch A, Addor MC, Yamaguti PM, Safatle HP, Acevedo AC, Martelli-Junior H, Dos Santos Netos PE, Coletta RD, Gruessel S, Sandmann C, Ruehmann D, Langman CB, Scheinman SJ, Ozdemir-Ozenen D, Hart TC, Hart PS, Neugebauer U, Schlatter E, Houillier P, Gahl WA, Vikkula M, Bloch-Zupan A, Bleich M, Kitagawa H, Unwin RJ, Mighell A, Berdal A, Kleta R. Nephrocalcinosis (Enamel Renal Syndrome) Caused by Autosomal Recessive FAM20A Mutations. Nephron Physiol. 2012; 122:1–6. [PubMed: 23434854]

76. Xiao J, Tagliabracci VS, Wen J, Kim SA, Dixon JE. Crystal structure of the Golgi casein kinase. Proc Natl Acad Sci U S A. 2013; 110:10574–10579. [PubMed: 23754375]

77. Taylor SS, Kornev AP. Protein kinases: evolution of dynamic regulatory proteins. Trends Biochem Sci. 2011; 36:65–77. [PubMed: 20971646]

78. Schumacher MA, Piro KM, Xu W, Hansen S, Lewis K, Brennan RG. Molecular mechanisms of HipA-mediated multidrug tolerance and its neutralization by HipB. Science. 2009; 323:396–401. [PubMed: 19150849]

79. Pei J, Grishin NV. PROMALS3D: multiple protein sequence alignment enhanced with evolutionary and three-dimensional structural information. Methods Mol Biol. 2014; 1079:263–271. [PubMed: 24170408]

80. Schmidt, HA. Testing Tree Topologies. 2. Cambridge University Press; Cambridge, Place Published: 2009.

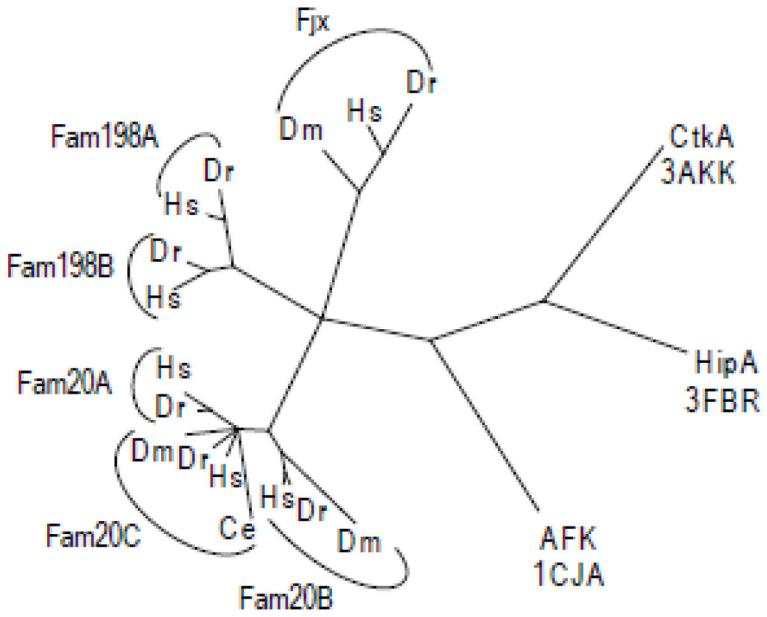

Figure 1. Evolution of Secretory Pathway Kinases

Fam20 sequences (Fam20A, Fam20B, Fam20C, Fjx, Fam198A, and Fam198B) were aligned using PROMALS [79]. Alignments of Fam20 sequences to the closest kinase structures (HipA, CtkA, and AFK) were guided by ceFam20 structure superpositions. Trees were built using Tree-Puzzle [80] using aligned superimposable sequence regions from Human (Hs), Fish (Dr), and Fly (Dm) representatives, as well as the structures.

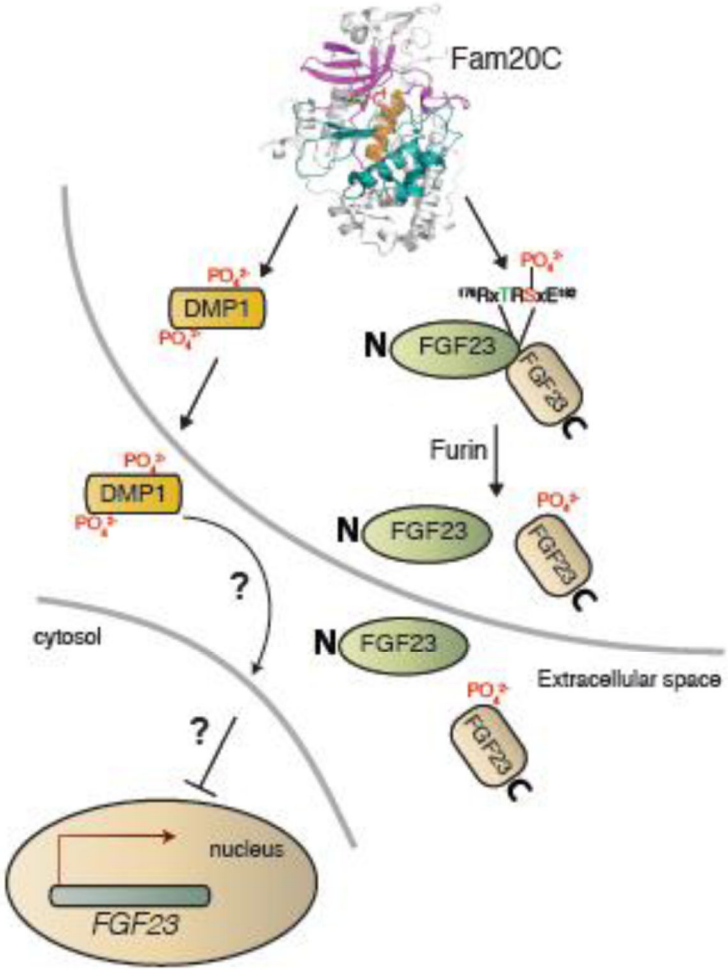

Figure 2. Regulation of FGF23 by Fam20C

Fam20C phosphorylates FGF23 at Ser^{180} within an important regulatory motif. This phosphorylation event prevents O-glycosylation of FGF23 at Thr^{178} by GalNAc-T3, which results in furin-dependent proteolysis and inactivation of FGF23. Fam20C may also regulate FGF23 expression indirectly, via phosphorylation of the SIBLING, DMP1. Phosphorylated DMP1 appears to negatively regulate FGF23 expression. However, the mechanism by which this occurs is currently unknown.

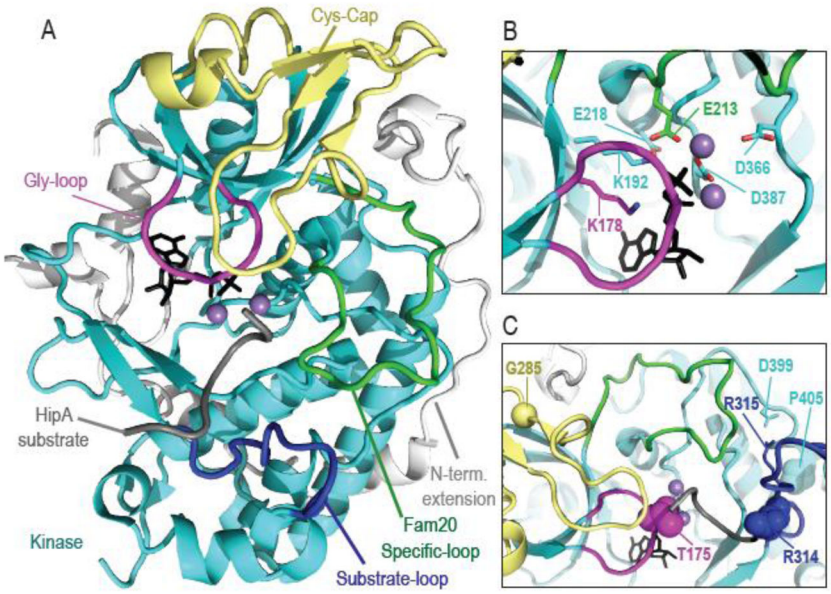

Figure 3. ***C. elegans*** **Fam20 Kinase Structure**

A) ceFam20C structure bound to Mn^{2+} (purple sphere) and ADP (black stick) [PDB: 4kqb] depicts canonical kinase domain (cyan) as well as Fam20-specific components: N-terminal extension (white), gly-loop (magenta), Cys-cap (yellow), Fam20-specific loop (green), and substrate-loop (blue). The ceFam20 kinase domain was superimposed with a peptide-bound HipA kinase [PDB:3fbr], and the HipA peptide substrate is displayed in cartoon (gray). **B)** ceFam20C active site colored as above displays conserved canonical active site residues (cyan stick), along with a conserved gly-loop residue (magenta stick) that binds phosphate, and a Fam20C-specific residue that orients Mn^{2+} (green stick). **C)** ceFam20C active site with disease related mutations depicted in spheres is colored as above. Mutations surround the superimposed peptide substrate (T175 and R314), help orient the substrate-loop (P405) through a Fam20 conserved salt-bridge (R315 and D399), or allow proper folding of the Cys-cap (G285).

Table 1

Secreted kinases in disease

Kinase	Substrate	Mutation	Disease	Reference
Fam20A	Unknown	G331D D403N L173R	Amelogenesis imperfecta Gingival hyperplasia Enamel Renal Syndrome	(*1–4*)
Fam20C	SIBLING FGF23	I258N;P496L; G280R;R408W; T268M;P328S; G379R; L388R; D451N; R549W;I246N; G266R;R558W; G379Q; I244N; P314S;G365Q; L374R;D437N; R535W	Raine syndrome Hypophosphatemia	(*5–6*) (*7–8*)
SGK196	α-dystroglycan	Q258R L137R	Congenital muscular dystrophy	(*9–10*)

References:

*1*Cho SH, *et al.* (2012) Novel FAM20A mutations in hypoplastic amelogenesis imperfecta. *Human mutation* 33(1):91–94.

*2*O'Sullivan J, *et al.* (2011) Whole-Exome sequencing identifies FAM20A mutations as a cause of amelogenesis imperfecta and gingival hyperplasia syndrome. *American journal of human genetics* 88(5):616–620.

*3*Wang SK, *et al.* (2014) FAM20A mutations associated with enamel renal syndrome. *Journal of dental research* 93(1):42–48.

*4*de la Dure-Molla M, *et al.* (2014) Pathognomonic oral profile of Enamel Renal Syndrome (ERS) caused by recessive FAM20A mutations. *Orphanet journal of rare diseases* 9:84.

*5*Simpson MA, *et al.* (2007) Mutations in FAM20C are associated with lethal osteosclerotic bone dysplasia (Raine syndrome), highlighting a crucial molecule in bone development. *American journal of human genetics* 81(5):906–912.

*6*Simpson MA, *et al.* (2009) Mutations in FAM20C also identified in non-lethal osteosclerotic bone dysplasia. *Clinical genetics* 75(3):271–276.

*7*Rafaelsen SH, *et al.* (2013) Exome sequencing reveals FAM20c mutations associated with fibroblast growth factor 23-related hypophosphatemia, dental anomalies, and ectopic calcification. *Journal of bone and mineral research : the official journal of the American Society for Bone and Mineral Research* 28(6):1378–1385.

*8*Takeyari S, *et al.* (2014) Hypophosphatemic osteomalacia and bone sclerosis caused by a novel homozygous mutation of the FAM20C gene in an elderly man with a mild variant of Raine syndrome. *Bone* 67:56–62.

*9*Jae LT, *et al.* (2013) Deciphering the glycosylome of dystroglycanopathies using haploid screens for lassa virus entry. *Science* 340(6131):479–483.

*10*Yoshida-Moriguchi T, *et al.* (2013) SGK196 is a glycosylation-specific O-mannose kinase required for dystroglycan function. *Science* 341(6148):896–899.