Cytoskeletal organization of the osteoclast (OC), which is central to the capacity of the cell to resorb bone, is induced by occupancy of the αvβ3 integrin or the macrophage colony-stimulating factor (M-CSF) receptor c-Fms. In both circumstances, the tyrosine kinase Syk is an essential signaling intermediary. We demonstrate that Cbl negatively regulates OC function by interacting with SykY317. Expression of nonphosphorylatable SykY317F in primary Syk−/− OCs enhances M-CSF- and αvβ3-induced phosphorylation of the cytoskeleton-organizing molecules, SLP76, Vav3, and PLCγ2, to levels greater than wild type, thereby accelerating the resorptive capacity of the cell. SykY317 suppresses cytoskeletal organization and function while binding the ubiquitin-protein isopeptide ligase Cbl. Consequently, SykY317F abolishes M-CSF- and integrin-stimulated Syk ubiquitination. Thus, Cbl/SykY317 association negatively regulates OC function and therefore is essential for maintenance of skeletal homeostasis.

OCs are multinucleated cells generated by fusion of mononuclear progenitors of the monocyte/macrophage family under the aegis of M-CSF and receptor activator of nuclear factor κB ligand (RANKL) (1). Upon mineralized matrix recognition, the OC polarizes its fibrillar actin, eventuating in the formation of an acidified extracellular microenvironment that degrades bone. Failure to undergo this polarization results in OC hypofunction and consequently in varying degrees of osteopetrosis (2). Integrins are transmembrane α/β heterodimers that mediate cell-cell and cell-matrix interactions and generate intracellular signals when occupied by ligands (3). The integrin, αvβ3, is expressed by OCs, and binding of this complex to bone is pivotal to the resorptive process (4).

M-CSF recognizes its transmembrane receptor tyrosine kinase, c-Fms, and induces receptor autophosphorylation at seven tyrosine residues within the cytoplasmic domain (5). Several Src homology-2 domain-containing molecules are recruited to the phosphotyrosine residues upon M-CSF binding and initiate signaling cascades that lead to cytoskeletal organization, survival, and proliferation of OC lineage cells (5–7). Both the αvβ3 integrin and M-CSF are important regulators of OC actin remodeling (4, 6, 8).

Syk is a 72-kDa nonreceptor tyrosine kinase, which mediates αvβ3- and c-Fms-induced OC cytoskeletal organization and function in a phosphorylation-dependent manner via a process involving activation of associated adaptor proteins, such as SLP-76 and Vav3 (9, 10). A number of Syk tyrosine residues undergo phosphorylation following engagement of the integrin and Fcγ receptor in immune (11) and mast cells (12). Three conserved tyrosine residues in the Syk linker region, namely Tyr317, Tyr342, and Tyr346, lie within consensus sequences for recognition by Src homology 2 domains, suggesting they transduce signals. Although phospho-SykY342 and phospho-SykY346 may serve as positive signaling regulators (12–14), phosphorylation of SykY317 creates a binding site for c-Cbl, an E3 ubiquitin ligase proposed to prompt ubiquitination and subsequent degradation of Syk (15, 16). Hence, SykY317 is a candidate negative regulatory site, but its role in OC function and/or differentiation is unknown.

Cbl is a 120-kDa protein that is tyrosine-phosphorylated following activation by growth factors, cytokines, and integrins. It has two distinct but related activities, serving both as an adaptor protein (17, 18) and E3 ubiquitin ligase (19, 20). Cbl functions principally as an adaptor in OCs by participating in signaling complexes that are important in the assembly and remodeling of the actin cytoskeleton (18, 21). In other cell types, Cbl is also a negative regulator of receptor and nonreceptor tyrosine kinases, as it promotes their degradation (22). OCs and their precursors express c-Cbl and another family member Cbl-b that compensates for the absence of c-Cbl (23, 24). As combined deletion of both isoforms eventuates in early embryonic lethality (24), it is not clear if c-Cbl functions as an E3 ubiquitin ligase in OCs. We establish that c-Cbl, recognizing SykY317, prompts the ubiquitination of the kinases thereby arresting activation of cytoskeleton-organizing molecules and thus OC function. The Cbl-SykY317 complex is therefore important in maintenance of normal skeletal mass.

**EXPERIMENTAL PROCEDURES**

Mice—Syk−/− (129/SV background) mice were described previously (25). Because of perinatal lethality of Syk−/− mice, we generated bone marrow chimeras by transplanting Syk−/−
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fetal liver cells into lethally irradiated WT recipients (9). Chimeras were used as a source of bone marrow macrophages (BMMs) 4–8 weeks after bone marrow transplantation.

All mice used in these experiments were 6–8 weeks old and housed in the animal care unit of Washington University School of Medicine, where they were maintained according to the guidelines of the Association for Assessment and Accreditation of Laboratory Animal Care. All animal experimentation was approved by the Animal Studies Committee of Washington University School of Medicine.

Reagents—Recombinant murine M-CSF was obtained from R & D Systems (Minneapolis, MN). Glutathione S-transferase (GST)-RANKL was expressed in our laboratory as described (26). The source of antibodies is as follows: mouse anti-Syk monoclonal antibody from Abcam (Cambridge, MA); anti-phosphotyrosine monoclonal antibody 4G10 and rabbit anti-Vav3 from Upstate (Charlottesville VA); monoclonal antibody 327, directed against the c-Src protein, were gifts of Dr. A. Shaw (Department of Pathology, Washington University School of Medicine, St. Louis, MO); rabbit anti-Src p-Y416 antibody, rabbit anti-phospho-Cbl (Y774) antibody, rabbit anti-M-CSF-76 antibody, and rabbit anti-phospho-PLCγ2 antibody from Cell Signaling (Beverly MA); goat anti-PLCγ6 as described previously (27); rabbit anti-Syk (N-19), mouse anti-ubiquitin, rabbit anti-Cbl, and mouse anti-PLCγ2 antibody from Santa Cruz Biotechnology (Santa Cruz, CA). The plasmid transfection reagent FuGENE 6 was purchased from Roche Applied Science. All other chemicals were obtained from Sigma.

Macrophage Isolation and OC Culture—Primary BMMs were prepared as described previously (28) with slight modifications. Marrow was extracted from femora and tibiae of 6–8-week-old mice with minimum Eagle’s α-medium and cultured in minimum Eagle’s α-medium containing 10% inactivated fetal bovine serum, 100 IU/ml penicillin, and 100 μg/ml streptomycin (α-10 medium) with 1:10 CMG condition media (29) on bacterial plastic dishes. Cells were incubated at 37 °C in 6% CO2 for 3 days and then washed with PBS and lifted with 1× trypsin/EDTA (Invitrogen) in PBS. A total of 5 × 105 cells were cultured in 200 μl of minimum Eagle’s α-medium containing 10% heat-inactivated fetal bovine serum with 100 ng/ml GST-RANKL and 30 ng/ml mouse recombinant M-CSF in 96-well tissue culture plates, some containing sterile bone slices. Cells were fixed and stained for tartrate-resistant acid phosphatase (TRAP) activity after 6 days in culture, using a commercial kit (LI-COR Biosciences).

Staining of Actin Ring and Bone Resorptive Pits—For actin ring staining, cells were cultured on bovine bone slice in the presence of M-CSF and RANKL for 6 days at which time cells were fixed in 4% paraformaldehyde, permeabilized in 0.1% Triton X-100, rinsed in PBS, and immunostained with Alexa 488 phalloidin (Molecule Probes). To quantitate resorption lacunae, cells were removed from bone slices with mechanical agitation. Bone slices were incubated with peroxidase-conjugated wheat germ agglutinin (Sigma) for 1 h and stained with 3,3′-diaminobenzidine (Sigma).

Media C-terminal Cross-linking Telopeptide of Bone Collagen (CTx) Assay—BMMs were cultured on bovine bone slice in 96-well plates with RANKL and M-CSF for 6 days. α-10 medium was changed 1 day before harvesting. Medium CTx concentration was determined using a CrossLaps for Culture ELISA kit (Nordic Bioscience Diagnostics A/S, Herlev, Denmark).

Syk Kinase Assay—BMMs were cultured on tissue culture plates with RANKL and M-CSF for 3 days. Cells were lifted with 0.1% EDTA and then replated on vitronectin-coated plates for 30 min. Cells were lysed, and Syk kinase activity was tested using Omnia plate-based assay kit (Invitrogen).

Plasmids and Retroviral Transduction—Wild type human Syk cDNAs, a gift from Dr. Sanford Shattil (University of California, San Diego), were subcloned into the BamHI and Xhol sites of a pMX retroviral vector in which the puromycin resistance sequence was replaced with one coding for blasticidin resistance. Y317F mutant was generated using the Quick-Change® site-directed mutagenesis kit (Stratagene, La Jolla, CA). WT and Y317F mutant Syk cDNA was transfected transiently into Plat-E packaging cells using FuGENE 6 transfection reagent (Roche Applied Science). Virus was collected 48 h after transfection. BMMs were infected with virus for 24 h in the presence of 100 ng/ml M-CSF and 4 μg/ml Polybrene (Sigma). Cells were selected in the presence of M-CSF and 1 μg/ml blasticidin (Calbiochem) for 3 days prior to use as OC precursors.

Western Blotting and Immunoprecipitation—Culture cells were washed twice with ice-cold PBS and lysed in RIPA buffer containing 20 mM Tris, pH 7.5, 150 mM NaCl, 1 mM EDTA, 1 mM EGTA, 1% Triton X-100, 2.5 mM sodium pyrophosphate, 1 mM β-glycerophosphate, 1 mM Na3VO4, 1 mM NaF, and 1× protease inhibitor mixture (Roche Applied Science). After incubation on ice for 10 min, cell lysates were clarified by centrifugation at 15,000 rpm for 10 min. Forty micrograms of total lysates were subjected to 8% SDS-PAGE and transferred onto nitrocellulose (Whatman, Maidstone, UK). Immunoblotting was carried out as described previously (30). Membranes were incubated with the Odyssey infrared imaging system (LI-COR Biosciences).

RESULTS

SykY317F Enhances OC Spreading—SykY317 is phosphorylated, in the OC, in response to αvβ3 integrin engagement (9) or M-CSF stimulation (10). To assess the role of SykY317 in OC function, we retrovirally transduced hemagglutinin-tagged SykWT and SykY317F into Syk−/− BMMs, which after 3 days of culture in M-CSF express equal amounts of the native and mutated protein (Fig. 1A).

Syk−/− BMMs differentiate normally into OCs, but the mutant polykaryons are dysfunctional as they fail to organize their cytoskeleton and optimally resorb bone (9). To assess the role of SykY317 in OC function, we treated SykWT and SykY317F BMMs with increasing doses of RANKL and M-CSF for 6 days (Fig. 1, B and C). Mirroring our previous data, WT Syk rescues the cytoskeletal abnormalities of Syk−/− OCs (9) and, in keeping with unaltered OC number in Syk−/− mice, does not affect...
osteoclastogenesis (Fig. 1B). Surprisingly, Syk<sup>Y317F</sup> not only rescues the cytoskeletal abnormalities of Syk<sup>−/−</sup> OCs (Fig. 1, B–D), but cells expressing the nonphosphorylatable mutant actually spread more effectively than WT (Fig. 1, B and E). The enhancement of size and spreading is particularly evident at lower concentrations of RANKL, indicating that Syk<sup>Y317F</sup> sensitizes OCs to the cytokine.

To ensure that these morphological abnormalities of Syk<sup>Y317F</sup> OCs do not reflect accelerated differentiation, we measured a series of markers of osteoclastogenesis in BMMs exposed to M-CSF and RANKL with time. Expression of characteristic osteoclastogenic proteins is not enhanced in Syk<sup>Y317F</sup> cells (Fig. 1F) nor are changes in specific intracellular signaling events that mediate OC differentiation (Fig. 1, G and H), namely RANKL-induced NF-κB, assessed by IκB-α phosphorylation and degradation, as well as c-Jun N-terminal kinase, ERK1/2, and p-38 phosphorylation. M-CSF-driven ERK1/2 and AKT phosphorylation are also normal in Syk<sup>Y317F</sup> OCs.

Syk<sup>Y317F</sup> Enhances OC Function—To further explore the cytoskeletal features of Syk<sup>Y317F</sup>-expressing OCs, we maintained BMMs on bone slices in M-CSF and RANKL for 6 days, in parallel with experiments depicted in Fig. 1. The actin cytoskeleton was visualized with fluorescein isothiocyanate-phalloidin. In correlation with their size, Syk<sup>Y317F</sup> OCs on the resorptive substrate have enlarged actin rings (Fig. 2A), a critical hallmark of cytoskeletal organization. Establishing that the unusual cytoskeletal morphology of Syk<sup>Y317F</sup>-bearing OCs translates into the capacity of the cells to degrade bone, their ability to form resorptive pits is significantly increased, relative to WT, particularly at low dose RANKL (Fig. 2, B and C). Most importantly, medium CTx of Syk<sup>Y317F</sup> OCs on bone is increased 3-fold relative to WT (Fig. 2D). The fact that mutant and WT OC numbers are indistinguishable (Fig. 1D) establishes that the enhanced bone degradation reflects accelerated resorptive activity per cell and not stimulated osteoclastogenesis.

Kinase Activity of Syk<sup>Y317F</sup> Is Not Increased—The capacity of Syk to organize the OC cytoskeleton depends upon its phosphorylation, mediated in the context of integrin activation by c-Src, and in response to M-CSF by autophosphorylation (9, 10). Thus, the stimulated function of Syk<sup>Y317F</sup>-bearing OCs might reflect enhanced c-Src and/or Syk activation. To deter-

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**FIGURE 1.** Syk<sup>Y317F</sup> mutation does not affect OC differentiation. Syk<sup>−/−</sup> BMMs were transduced with either WT Syk, Syk<sup>Y317F</sup>, or empty vector (Vect). A, Syk expression by transduced Syk<sup>−/−</sup> BMMs was determined by immunoblot. B, BMMs were cultured with M-CSF (30 ng/ml) and increasing amounts of RANKL (RL) for 6 days after which the cells were stained for TRAP activity. Spread OCs (arrows), which are absent in vector-transduced cells, appear at lower RANKL doses and are more abundant in those expressing Syk<sup>Y317F</sup> than the WT construct. C, similar intensity of TRAP activity (red reaction product) in wells containing OCs generated from Syk<sup>Y317F</sup> and WT BMMs. D, histological quantification of the numbers of OCs/well (more than three nuclei per cell) generated from Syk<sup>Y317F</sup> and WT BMMs. Inset shows characteristic spread and nonspread OCs, respectively. (***, p < 0.001.) E, transduced Syk<sup>−/−</sup> BMMs were cultured with RANKL (100 ng/ml) and M-CSF (30 ng/ml), with time. OC differentiation markers were determined by immunoblot. Actin serves as loading control. CathK, cathepsin K. G and H, transduced Syk<sup>−/−</sup> BMMs, cultured in 50 ng/ml M-CSF for 3 days, were serum- and cytokine-starved overnight. The cells were then exposed to either 100 ng/ml M-CSF (G) or 100 ng/ml RANKL (H) with time. Signaling molecules were identified by immunoblotting. Actin serves as a loading control.
mine whether integrin-induced activity of the two kinases is enhanced in mutant osteoclastic cells, we cultured WT- and SykY317F-expressing BMMs in RANKL and M-CSF. After 3 days, the cells were lifted and replated on the αvβ3 ligand, vitronectin, or maintained in suspension. As seen in Fig. 3A, integrin-induced Syk phosphorylation is unaltered by the Y317F mutation. Similarly, αvβ3-stimulated c-Src activity, as manifest by its Tyr416 phosphorylation and total protein phosphorylation in SykY317F-expressing OCs is indistinguishable from WT (Fig. 3B). Like αvβ3 occupancy, c-Fms activation prompts similar Syk (Fig. 3C) and total protein (Fig. 3D) phosphorylation in WT and mutant Syk-expressing cells. Furthermore, the Y317F mutant does not alter Syk kinase activity induced by αvβ3 occupancy (Fig. 3E).

SykY317F Super-activates Cytoskeleton-organizing Signaling Molecules—Syk is an intermediary in the αvβ3- and c-Fms-activated cytoskeletal organizing pathway in the OC, which involves target signaling molecules, including the guanine nucleotide exchange factor Vav3 (9). We therefore reasoned that the enhanced cytoskeleton-organizing capacity of SykY317F should be reflected by increased activity of Vav3. In fact, whether stimulated by integrin or c-Fms occupancy, Vav3 phosphorylation is enhanced in mutant osteoclastic cells (Fig. 4, A and B). PLCγ2 (30, 31) and SLP-76 (32) are also integrin- and M-CSF-induced OC cytoskeleton-organizing signaling molecules, which in other cells are activated by Syk (33, 34). Like Vav3, phosphorylation of both molecules, whether induced by αvβ3 or c-Fms occupancy, is increased in SykY317F OCs (Fig. 4, C–F). Thus, augmented OC function in a circumstance wherein SykY317F is not phosphorylated reflects activation of cytoskeleton-organizing molecules.

SykY317F Inhibits Cbl-mediated Ubiquitination—The unaltered kinase capacity of SykY317F suggests its enhanced net activity, at least in part, reflects increased abundance. In other cell types, phosphorylated SykY317F binds c-Cbl, which functions as E3 ligase, ultimately promoting Syk ubiquitination and degradation. Thus, failure of SykY317F to undergo phosphorylation may prolong the half-life of the protein. To address this hypothesis, in OCs, we first established that αvβ3 engagement phosphorylates SykY317F (Fig. 5A). Furthermore, whereas WT Syk/Cbl association is enhanced by αvβ3 occupancy, the same is not true in the context of SykY317F, a circumstance in which other Syk tyrosines are phosphorylated (Fig. 5B, and data not
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We have shown that Syk<sup>Y317F</sup> fails to recognize Cbl, the mutated kinase does not alter total Cbl tyrosine phosphorylation or that of Cbl<sup>Y744</sup>, one of the major sites of tyrosine phosphorylation (Fig. 5, C–E). Thus, Cbl phosphorylation does not depend on its recognition of Syk.

Because Cbl is an E3 ligase, its interaction with Syk may yield ubiquitination and consequent degradation of the tyrosine kinase. We therefore asked if αvβ3 or c-Fms ligation induces Syk ubiquitination and if so is the event altered by Syk<sup>Y317F</sup>. To this end, we pretreated WT and Syk<sup>Y317F</sup> pre-OCs with the proteasome inhibitor, MG-132, for 1 h before exposure to M-CSF. As shown in Fig. 6A, the cytokine induces ubiquitination of WT Syk (and/or associated proteins), which is totally arrested in the presence of Syk<sup>Y317F</sup>. Syk<sup>Y317F</sup> also dampens αvβ3-induced Syk ubiquitination (Fig. 6B).

To determine the potential impact of the cytokine on Syk degradation, we treated WT and Syk<sup>Y317F</sup> marrow macrophages with M-CSF and RANKL for 3 days to commit them to the OC phenotype. The cells were exposed to cycloheximide in the absence of cytokines for 1 h to arrest protein synthesis. The cells were treated with M-CSF, and the quantity of WT and mutated Syk was determined by immunoblot during the next 4 h. Consistent with its activity being unaltered in nonstimulated cells (Fig. 4), basal amounts of the tyrosine kinase are similar in those bearing WT and Y317F mutated Syk (Fig. 6C). On the other hand, M-CSF promotes progressive degradation of WT Syk but not Syk<sup>Y317F</sup>. Interestingly, degradation of Syk is apparent prior to detectable ubiquitination.

DISCUSSION

All forms of pathological bone loss, including those attending autoimmune osteolysis, represent enhanced resorption relative to formation. In most circumstances, accelerated skeletal degradation reflects a combination of increased OC number and activity, the latter typically associated with cytoskeletal reorganization. In this circumstance, the cell polarizes to form actin rings and a ruffled membrane, both participating in delivery of matrix-degrading molecules into the resorptive microenvironment between bone and the juxtaposed plasma membrane (35).

Although the general morphological features of OC polarization have been long appreciated, insights into the relevant molecular mechanisms are recent. Matrix-derived signals, mediated via the αvβ3 integrin and M-CSF, are particularly important in organizing the cytoskeleton of the resorptive cell. In fact, the integrin and cytokine share many components of a canonical signaling pathway eventuating in OC polarization and bone degradation. This signaling complex includes the
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**A**

**B**

**C**

FIGURE 6. Syk_{Y317F} inhibits Syk ubiquitination and degradation. Syk−/− BMMs, transduced with either WT Syk or Syk_{Y317F}, were cultured with RANKL and M-CSF for 3 days. A, cells were pretreated with proteasome inhibitor MG-132 (10 μM) for 1 h and then stimulated with M-CSF with time. Syk immunoprecipitates were immunoblotted for ubiquitin (Ub). B, cells, lifted and maintained in suspension in the presence MG-132 (10 μM) for 1 h, were retained in suspension (S) or plated to vitronectin (A) for 3 h. Syk immunoprecipitates were immunoblotted for ubiquitin. C, cytokine- and serum-starved cells were pretreated with the protein synthesis inhibitor cycloheximide (20 μg/ml). After 20 min, M-CSF was added and Syk immunoblotted with time. Actin serves as loading control. Numbers represent densitometric analysis related to 0 h in each group. p-Y, phosphorylated tyrosine.

ITAM proteins, Dap12 and FcγRγ, Vav3, the SLP adaptor proteins, and the small GTPase, Rac (9, 10, 36). Interestingly, inactivation of any of these complex-residing proteins yields “created-appearing” OCs that fail to spread.

c-Src is also a key component of both adhesion- and cytokine-stimulated cytoskeleton organization associating with the β3 integrin subunit cytoplasmic domain as well as that of c-Fms (8, 37). In fact, until the recent discovery of the role of Syk, c-Src was the only nonreceptor tyrosine kinase with an established role in the OC (38). Like c-Src, Syk is phosphorylated downstream of the integrin and c-Fms, but the mechanism of activation differs in each circumstance. Whereas Syk is phosphorylated by c-Src in the context of αβ3 (9), it undergoes autophosphorylation upon M-CSF exposure (10). Regardless of mechanism, activated Syk stimulates the distal cytoskeleton-organizing signaling complex.

Syk contains three tyrosine residues in its linker region separating the catalytic and Src homology 2 domains of the molecule. Although each is phosphorylated upon B-cell receptor engagement, the consequences differ. Syk_{Y342} and Syk_{Y346} phosphorylation activate phospholipase C-γ and Ca^{2+} mobilization (39). Similarly, mutation of Syk_{Y342} and Syk_{Y346}, in combination, diminishes mast cell degranulation (12). On the other hand, Syk_{Y317} phosphorylation is generally inhibitory of events emanating from the B-cell receptor (12, 16, 40). Interestingly, Syk_{Y317} is phosphorylated under the aegis of the Src family kinase, Lyn, which we have established is also anti-resorptive (41).

Syk-deficient OCs fail to spread, lack actin rings, and have attenuated bone resorptive activity. As expected, all three defects are rescued by expressing WT Syk in cells lacking the tyrosine kinase. The novel feature of this study is that, as in B-cells and mast cells, Syk_{Y317} also blunts the OC. Alternatively, inhibition of phosphorylation of the residue, which occurs upon αβ3 occupancy, ”super-rescues” the cytoskeletal and resorptive dysfunction of Syk−/− OCs as manifest by spreading, actin ring formation, and bone degradation. These cells are reminiscent of those generated in the absence of SHIP1, another molecule that physiologically restrains OC function (42). The fact that the stimulatory effect Syk_{Y317F} exerts on resorptive activity reflects accelerated activity and not increased OC number is consonant with normal expression and activation of differentiation and immediate signaling molecules, respectively.

SLP-76, an adaptor protein lacking intrinsic enzyme activity, plays a key role in T-cell receptor-derived signals, including those that regulate the actin cytoskeleton (43). SLP-76 is phosphorylated by Syk in a number of cells, and we find the same holds following αβ3 engagement or M-CSF treatment of OCs. In keeping with the negative regulatory function of Syk_{Y317}, SLP-76 is hyper-phosphorylated in Syk_{Y317F}-bearing cells. In consequence, Vav3, which is a component of the cytoskeleton-organizing complex recruited by activated SLP-76, also undergoes enhanced phosphorylation in the mutant OCs. Thus, Syk_{Y317} restrains OC cytoskeletal organization by dampening activation of effector molecules.

Cbl family proteins are evolutionarily conserved negative regulators that associate with protein-tyrosine kinases upon their activation. Cbl recognizes, ubiquinates, and negatively regulates Syk in T-cells and mast cells and does so by binding to phosphorylated Syk_{Y317} (16, 22, 44, 45). Having defined the role of WT Syk in OCs (9), these observations prompted us to determine whether the bone resorptive function of the kinase is altered by preventing Tyr_{Y317} phosphorylation. Similar to its association with other αβ3-associated proteins in OCs, Cbl recognizes Syk upon integrin activation (21) in this circumstance by binding Tyr_{Y317}. Although the nonphosphorylatable Y317F mutant blocks Syk/Cbl association, it does not alter activation of either molecule. This observation, taken with the normal Cbl phosphorylation extent in Syk−/− OCs (data not shown), indicates Syk and Cbl activation are independent events.

On the other hand, the Y317F mutant profoundly reduces Syk ubiquitination, particularly in the context of M-CSF.
Because Syk degradation is arrested in cells bearing the mutated tyrosine kinase, a reasonable conclusion would hold that the “super-spread” and hyper-resorptive phenotype of SykT317F OCs reflects protection of the tyrosine kinase from Cbl-mediated proteosomal degradation. This thesis, however, is challenged by an apparent temporal inconsistency between suppressed ubiquitination of SykT317F and its increased abundance in M-CSF-treated cells (Fig. 6). Specifically, degradation of WT Syk is detected with 1 h of exposure to the cytokine, although SykT317F is protected. In contrast, ubiquitination of WT Syk is apparent only after 3 h, a discrepancy that may represent distinct sensitivities of the two assays. This posture is in keeping with our observation that coincident with Cbl phosphorylation, an array of proteins absent change in Syk quantity (23). Thus, cytoplasmic Cbl activity, in other cells, prompts phosphorylation of a similar array of proteins, whether induced by M-CSF or integrin liganding (Fig. 4). This observation may, however, reflect the fact that arrest of Syk Tyrosine 317 Regulates Osteoclasts

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