that Derivery et al. (2009) observed loss of Arp2/3 and F-actin from endosomes upon WASH depletion, whereas Gomez and Billadeau (2009) did not. Again, this could be due differences in experimental procedures, and resolution will require further coordinated experiments.

Regardless of any outstanding differences, these two papers break significant new ground and expand our understanding of Arp2/3 activation and endosome sorting. The emerging picture of NPF activation of Arp2/3 is one in which diverse NPFs respond to various upstream signals to generate branched actin networks in many cellular locations. One thing that will be helpful for the field going forward is to adopt the new consensus nomenclature for WASH's conserved N-terminal domains proposed in these two papers (Derivery et al., 2009, and Gomez and Billadeau, 2009). The original names of WASH's N-terminal domains, the WHD1 and 2 domains, were confusingly similar to the names previously used for WASP and SCAR/WAVE's distinct N-terminal domains. The new proposed names for these domains are the *WA*SH *h*omology *d*omain 1 (WAHD1) and *t*ubulin-*b*inding *r*egion (TBR). Collectively, the N-terminal region containing these domains is referred to as *WA*SH *h*omology *d*omain (WAHD). Consensus in the field now could save a great deal of confusion in the future.

With all new discoveries, unanswered questions pile up quickly, and WASH is no exception. Beyond sorting out the composition of the multiprotein complex that WASH participates in, understanding its interactions with other proteins and lipids will require many new experiments. For example, WASH interacts with tubulin/microtubules (Derivery et al., 2009; Gomez and Billadeau, 2009; Liu et al., 2009), but it is not clear how this interaction relates to the Arp2/3 activating function. *Drosophila wash* has also been shown to interact with the GTPase Rho (Liu et al., 2009). Is this interaction conserved and could mammalian RhoB, a GTPase linked to endosome trafficking, be an upstream regulator of WASH? Finally, loss of WASH function leads to tubulation, whereas overexpression of the Golgi-localized WHAMM leads to this phenotype. How can these related NPFs produce opposite phenotypes? We will have an exciting and busy couple of years answering these and other questions about WASH.

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Developmental ECM Sculpting: Laying It Down and Cutting It Up

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In mammals, proteolytic remodeling of the embryonic extracellular matrix (ECM) controls morphogenesis, but the key players remain elusive. Two recent reports identify new roles for metalloproteinases belonging to the MT-MMP and ADAMTS families in branching morphogenesis and interdigital web regression.

During development, the extracellular matrix (ECM)—the composite of structural proteins, proteoglycans, and glycosaminoglycans that maintains tissue architecture—undergoes complex, cell-mediated remodeling in order to accommodate the morphogenetic programs that give rise to structures ranging from branching glands to digits. While temporal and spatial changes in ECM biosynthesis, as well as the application of cell-derived mechanical forces, all participate in sculpting the final body plan, increasing evidence points to additional—and important—roles for proteolytic enzymes. In terms of developing mechanistic insight into this process, considerable intellectual and technical hurdles must first be negotiated, especially with regard to mammalian developmental programs. First, the mammalian genome encodes hundreds of proteinases capable of operating in the extracellular milieu, giving rise to an almost mind-boggling array of possible schemes

for enzymatic redundancy, enzymatic compensation, and adaptive development (Page-McCaw et al., 2007; Rowe and Weiss, 2009). Second, though the characterization of some developmental processes may lend themselves to in vitro modeling, recent—and appropriate—criticism has been leveled against the ability of standard 2D culture systems to recapitulate cell functions that normally operate within a 3D ECM whose complex composition and structural properties cannot be duplicated readily in vitro (Yamada and Cukierman, 2007; Rowe and Weiss, 2008). Against this backdrop of concerns, what is a developmental biologist to do? Two recent publications describe successful efforts to tackle these issues by using intact tissues to study classic, but distinct, examples of mammalian histogenesis: submandibular gland branching morphogenesis (Rebustini et al., 2009) and interdigital web regression during limb morphogenesis (McCulloch et al., 2009). In each case, redundant sets of metalloproteinases, belonging respectively to the MMP (matrix metalloproteinase) and ADAMTS (a disintegrin and metalloproteinase with thrombospondin repeats) families, are shown to drive morphogenesis by cleaving ECM substrates as a means for not only sculpting tissue architecture but also for generating bioactive ECM fragments critical to the respective developmental program.

Prior to the inception of salivary gland branching morphogenesis, the oral epithelium sits atop the basement membrane (BM), a specialized form of ECM that lies beneath all epithelial cell layers. Approximately 100–300 nm thick, the BM is composed largely of a mixture of specialized glycoproteins and proteoglycans (e.g., laminins, nidogens, and sulphated proteoglycans) whose mechanical integrity is determined by an intertwined and covalently crosslinked network of type IV collagen heterotrimers (Rowe and Weiss, 2008). This sheet-like network of type IV collagen—noncovalently decorated with laminin polymers and structural proteoglycans—constitutes the core element of the BM that separates overlying epithelium from underlying stromal tissues. Hence, as salivary epithelial cells receive signals to initiate branching, they must not only proliferate, activate motile machinery, and orchestrate the formation of an elongating hollow tube that juts deeply into the stromal space, but do so while continuously assembling and depositing a new BM scaffolding. Based largely on analyses of BM remodeling in model organisms (Page-McCaw et al., 2007), it has been presumed that branching morphogenesis in mammals would somehow coordinate the removal of "old" BM with the deposition of "new" BM. But what proteinases might initiate BM remodeling and how might this process be orchestrated with morphogenesis?

Using explants of mouse submandibular glands, which undergo a visually striking program of branching morphogenesis ex vivo, Rebustini and colleagues in a recent issue of *Developmental Cell* focused their attention on a small group of membrane-tethered matrix metalloproteinases, termed the MT-MMPs (Rebustini et al., 2009). While multiple MMPs in this 24-member family have been linked to BM remodeling (largely in the context of tumor invasion and metastasis; Rowe and Weiss, 2009), the secreted MMPs do not appear to affect branching in this context. The authors therefore focused on MT2-MMP, the dominant MT-MMP family member expressed in the branching epithelium, and found that it is required for branching morphogenesis. siRNA directed against mesenchymal MT3-MMP did not impact branching but elicited a dramatic increase in MT2-MMP expression, highlighting the existence of potentially important crosstalk between the proteolytic machinery of the epithelial and mesenchymal compartments. Finally, despite the restriction of MT1-MMP expression to the gland mesenchyme, its silencing also decreased branching. Stromal cells (e.g., fibroblasts) lying within the mesenchyme may be found in close association with the BM, raising the interesting possibility that ECM remodeling during branching occurs as a consequence of a combined assault on both the apical and basal faces of the BM.

Given the key role that the type IV collagen network plays in maintaining the structural integrity of the BM, the collagenous backbone seemed a likely substrate for these MT-MMP functions (Hotary et al., 2006). Furthermore, in a theme repeated with increasing frequency in ECM biology (Page-McCaw et al., 2007), Rebustini et al. (2009) demonstrate that cryptic activities embedded within intact type IV collagen molecules are unmasked as a consequence of MT-MMP-dependent hydrolysis, allowing type IV fragments to initiate novel signal transduction cascades critical to the morphogenetic program. While these intriguing results await further confirmation in MT2-MMP gene-targeted and MT1-MMP/MT2-MMP double-null mice, the notion that MT-MMPs play a necessary role in supporting branching morphogenesis by proteolytically remodeling the BM—altering both its structural properties while simultaneously generating promorphogenic degradation products—provides exciting insights into this complex program.

While the report by Rebustini et al. (2009) focused on the proteolytic remodeling of the BM by the epithelium, McCulloch et al. (2009) in this issue of *Developmental Cell* now examine remodeling events within the stromal compartment of the ECM, where controlled tissue resorption allows new structure/function relationships to arise, i.e., the emergence of free digits as a consequence of interdigital web regression (McCulloch et al., 2009). Unlike the type IV collagen-rich BM, interdigital tissue of the embryonic stromal matrix is dominated by the glycosaminoglycan, hyaluranon, the embryonic proteoglycan, versican, and the glycoprotein, fibronectin. While screening for genes that modify the phenotype of ADAMTS20-deficient mice, the authors discovered that three ADAMTS family members, *Adamts5*, *Adamts9*, and *Adamts20* form a cooperative network in interdigital tissues that controls web regression (McCulloch et al., 2009). In considering potential ADAMTS substrates localized within web tissues, attention focused on the fact that multiple ADAMTS members are able to proteolyze chondroitin sulfate proteoglycans, including versican (McCulloch et al., 2009). Intriguingly, versican haploinsufficiency enhanced the interdigital defects observed in ADAMTS20-deficient mice, leading the authors to conclude that the ADAMTS-dependent *generation* of versican hydrolysis products, rather than the elimination of versican content per se, plays a required role in interdigital tissue regression. Indeed, when recombinant versican fragments known to be generated as a product of ADAMTS activity were inserted into the web tissues of ADAMTS mutant mice, a striking increase in interdigital apoptosis occurred. Presently, the precise role that ADAMTS-mediated versican proteolysis

plays in precipitating the resorption of the interdigital ECM remains to be determined as we do not know if (1) versican loss alone can initiate the collapse of the interdigit embryonic matrix, (2) versican-degradation products can trigger the expression of other matrix-degrading proteinases that precipitate ECM resorption, or (3) the substrate repertoire of the ADAMTS20/5/9 triad includes yet-to-be-identified substrates. Nevertheless, the presented data clearly highlight the importance of a novel, multiarmed ADAMTS-versican axis in orchestrating web regression and digit separation.

Finally, though both of these reports focus on the metalloproteinase-ECM axis, one cannot ignore the fact that neither branching nor resorption could be inhibited completely when all the "usual suspects" were targeted (McCulloch et al., 2009; Rebustini et al., 2009). Even in a metalloproteinase-centric world, BM remodeling likely includes ADAMTS members while stromal tissues also fall prey to the MT-MMPs (Chun et al., 2006; Kubota et al., 2008). Clearly, more work is needed to solve even the most basic of questions in developmental biology. Though the plaintive that "we know much more, but it's more complicated than we thought" is the bane of every public relations offensive to capture the public's eye, this truism remains the stuff of all serious scientific inquiry.

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