

MOLECULE PAGE

Mist1

Christopher Pin

Departments of Paediatrics and Physiology & Pharmacology,
Children's Health Research Institute, The University of Western Ontario
e-mail: cpin@uwo.ca

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Gene symbols: [Bhlha15](#)

1. General Function

MIST1 is a transcription factor belonging to the basic helix-loop-helix (bHLH) family of proteins that was discovered using a yeast one hybrid screening approach (13). The *Drosophila* homolog for MIST1 is dimmed (6) and the gene nomenclature in mice has recently changed from *Bhlhb8* (17) to *Bhlha15*. MIST1 is expressed in serous exocrine cells including acinar cells in the pancreas (16). Outside of the pancreas, MIST1 is expressed in the acinar cells of lacrimal, parotid and submandibular salivary glands, Chief cells of the stomach, alveolar cells of lactating mammary glands, and secreting cells lining the prostate and seminal vesicle (16). In the pancreas, MIST1 localizes to the nuclei of acinar cells (Figure 1A). No expression of MIST1 is observed in duct or centroacinar cells, although recent studies suggest that a population of islet cells may express MIST1 to very low levels (Pin and Konieczny, unpublished).

Functionally, MIST1 is a transcription factor belonging the B family of bHLH proteins, which are members of the family that exhibit a tissue-restricted pattern of expression. The MIST1 protein can form heterodimers with other bHLH

transcription factors as is typical for these proteins (12). However, unlike other bHLH proteins, MIST1 appears to preferentially work as a homodimer. This is based on evidence that over-expression of a dominant negative form of MIST1 leads to a similar phenotype in vivo as a targeted ablation of the *Mist1* gene (26). A number of genes including *Gjb1* (encodes Connexin32; (21), *Atp2c2* (encodes SPCA2; (5), p21 CIP1/WAF1 (8), *Rab3d* (9, 23), *Rab26* (23) and PHM (peptidylglycine alpha-hydroxylating monooxygenase; (15) have all been linked to MIST1's transcriptional ability. CAST (Cyclic Amplification and Selection of Targets) experiments have further defined the E box motif that MIST1 binds to as being CATATG (24).

While early studies identified MIST1 as a transcriptional repressor of myogenesis, terming the protein "muscle TWIST" (12), studies in the last 10 years have not identified an in vivo role for MIST1 in myogenesis. There is evidence that MIST1 is a target of the ER stress response transcription factor XBP1 (7), which also plays a physiological role in pancreatic acini. XBP1 promotes *Mist1* expression leading to loss of the differentiated state of myoblasts and beta cells in culture (1).

The *Mist1* gene is located on chromosome 5 in *mus musculus* and chromosome 7 in humans (18). The gene consists of two exons with the entire coding region contained in the second exon. The gene encodes for a 197 amino acid protein and, to date, only one isoform and no paralogues are known to exist. Dissection of the MIST1 protein has identified additional motifs outside of the basic helix-loop-helix domain known to allow for DNA binding and dimerization to other bHLH proteins (12, 13). The first 71 amino acids of the protein are believed to contain an active repressor domain based on Gal4 assays with different regions on the protein fused to a GAL4 DNA binding domain. A Q rich region exists in the 3' end of the protein although a function for this sequence has not been defined. The MIST1 protein does not contain a consensus nuclear localization signal or a transcription activation domain (TAD) and only the bHLH portion of the protein is required for transcriptional activity (24). However, MIST1 is located exclusively in the nucleus and can both promote and repress gene expression following co-transfection with luciferase reporter constructs in cell lines.

2. Mist1 in Pancreas

MIST1 is first expressed in pancreatic development around embryonic day (E) 10.25 (19). Based on this expression pattern it is likely expressed in both exocrine and endocrine precursors. Similar to PTF1a expression, the expression of MIST1 becomes restricted to acinar cells and is not expressed in duct cells or fibroblasts within the pancreas (Figure 1A). MIST1 is localized exclusively to nuclei and is expressed at easily detectable levels in adult tissue (16). The targeted ablation of the *Mist1* gene leads to numerous acinar cell phenotypes (19) although the hierarchy of events has still not been adequately established.

The initial study characterizing the phenotype of *Mist1*^{-/-} mice (19) identified a disruption in acinar cell organization (Figure 1C, E), premature activation of digestive enzymes, increased expression of pancreatitis associated proteins and loss of IP₃R3 expression. While *Mist1*^{-/-} mice are viable with no identified effects on reproduction or overall physiology, the pancreatic phenotype is progressive with increased tissue damage over time and pockets of acinar to duct cell metaplasia appearing in aged mice (19). This metaplasia was also observed in mice that over-express a basic domain mutant of MIST1 that acts as a dominant negative for transcriptional activity (26).

Since the initial study, a number of follow up studies have been published trying to identify a hierarchy of events within the phenotype as well as determine gene targets of MIST1 transcriptional activity. *Mist1*^{-/-} acini have a defect in mitochondrial localization and calcium movement (14), which is likely the underlying cause of decreased basal and regulated exocytosis exhibited by these cells. In addition to IP₃R3, a recent study (5) has identified the specific loss of novel form of *Atp2c2* (gene encoding secretory pathway Ca²⁺ ATPase 2; SPCA2), which has been implicated in store operated calcium entry (4). MIST1 also targets p21 pCIP/WAF causing growth arrest of acinar cells suggesting that, in the absence of MIST1 (8), the mature acinar cell phenotype is more plastic.

One outcome of the phenotypes observed in *Mist1*^{-/-} mice, is that they are more sensitive to pancreatic injury. A small subset of *Mist1*^{-/-} animals (<5%) undergoes spontaneous development of pancreatitis, and *Mist1*^{-/-} mice exhibit significantly more damage and a longer period of recovery following cerulein or arginine-induced pancreatitis (10). Induction of pancreatic injury by cerulein resulted in more necrosis, less apoptosis and higher amounts of circulating amylase compared to wild type counterparts. Microarray analysis revealed marked differences in the molecular profile of *Mist1*^{-/-} and wild type

mice before and after cerulein treatment. In particular, limited activation of the unfolded protein response (UPR), which is robustly observed in wild type tissue (11), is seen in the absence of MIST1. Likely this inability to trigger the UPR is due to an adaptive response of *Mist1*^{-/-} acini to a stressful cell environment, however, with *Mist1* identified as a transcriptional target of XPB1, a direct involvement of MIST1 in the UPR can't be ruled out. Because of these gaps in knowledge, further work is needed to delineate the targets of MIST1 transcriptional activity.

MIST1 is also expressed in AR42J cells and, to a lesser extent, 266.6 mouse acinar cells, but not in ARIP cells ((16); Pin and Mehmood, unpublished). MIST1 also reduces the ability of oncogenic Kras to promote pancreatic intraepithelial neoplasia (PanIN) (22). The absence of MIST1 in the presence of KrasG12D greatly accelerates PanIN formation and mice are not viable with almost a complete lack of acinar tissue replaced by ductal epithelium. These affects are reversed by the forced expression of MIST1 (22).

An absence of MIST1 also affects the differentiation and/or morphology of other serous exocrine cells include salivary glands (9), gastric epithelium (9, 20), mammary gland alveolar cells (25), seminal vesicle (17), and plasma cells (2, 3).

3. Tools for Study of Mist1

a. cDNA

cDNA Clones for mouse and rabbit MIST1 are available from Stephen Konieczny. A number of DNA constructs were generated in which regions of the MIST1 protein were mutated or deleted affecting MIST1 binding to DNA or other bHLH proteins (12, 24).

b. Antibodies

Rabbit antibodies raised against the C-terminal of mouse MIST1 have been used to identify MIST1 by western blotting, immunofluorescence and immunohistochemistry in AR42J cells (16), rat

(13) and pancreas (19). Additional antibodies have been made available by Santa Cruz Biotechnology as a IgG and stated to detect all isoforms of MIST1 of mouse, rat, and human origin. While these antibodies are believed to be ChIP ready, they have shown limited success in this assay to date.

c. Mouse lines

There are at least four reported transgenic and gene targeting mice that affect the expression of MIST1. Targeted ablation of the *Mist1* gene has been reported in which the *Mist1* coding region has been replaced by the *LacZ* reporter gene (19). A subsequent targeting event replaced the *Mist1* coding region with *cre recombinase*, thereby providing the ability to specifically delete floxed alleles in MIST1-expressing cells (22). This has allowed for lineage tracing of cells that express MIST1 transiently. A dominant-negative form of MIST1 in which the basic region was mutated to prevent DNA binding was expressed specifically in pancreatic acini using the *Elastase I* promoter leading to similar acinar cell disorganization as described in *Mist1*^{-/-} mice (26). Finally, a transgenic mice which allows for inducible expression of a myc-tagged MIST1 protein has been generated (5). These lines are all available from Stephen Konieczny.

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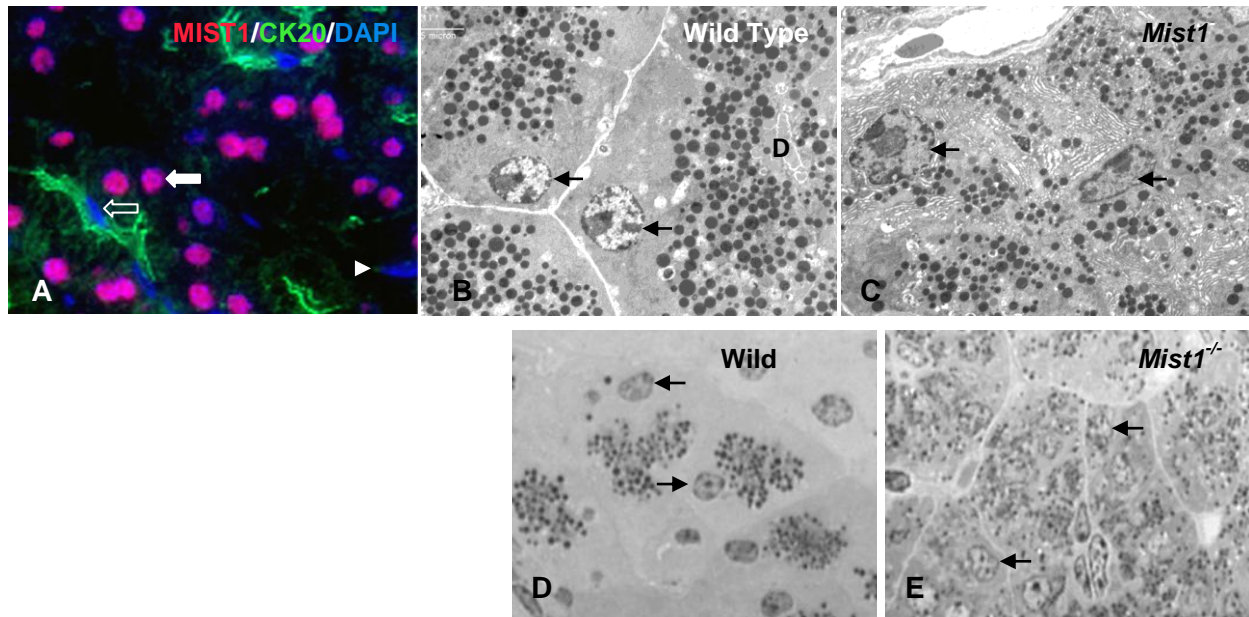


Figure 1. (A) Immunofluorescence for MIST1 (red) and cytokeratin 20 (green) on pancreatic tissue. Acinar cells (white arrow) express MIST1 while duct cells (open arrow) and fibroblasts (arrowhead) do not.

(B, C) High magnification electron micrographs and **(D, E)** methylene blue staining from wild type and *Mist1*^{-/-} mice. Zymogen granules are dispersed throughout *Mist1*^{-/-} acini. Nuclei are indicated by black arrows. EMs published in (19).

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