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MYST family histone acetyltransferases take center stage in stem cells and development

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Acetylation of histones is an essential element regulating chromatin structure and transcription. MYST (*M*oz, *Y*bf2/Sas3, *S*as2, *T*ip60) proteins form the largest family of histone acetyltransferases and are present in all eukaryotes. Surprisingly, until recently this protein family was poorly studied. However, in the last few years there has been a substantial increase in interest in the MYST proteins and a number of key studies have shown that these chromatin modifiers are required for a diverse range of cellular processes, both in health and disease. Translocations affecting MYST histone acetyltransferases can lead to leukemia and solid tumors. Some members of the MYST family are required for the development and self-renewal of stem cell populations; other members are essential for the prevention of inappropriate heterochromatin spreading and for the maintenance of adequate levels of gene expression. In this review we discuss the function of MYST proteins *in vivo*.

Keywords: Hbo1; Mof; Moz; Qkf/Morf; Tip60

Introduction

Chromatin structure regulates gene function in healthy adults, in disease and during development. The mechanisms involve, among others, covalent modifications of core histone residues such as acetylation and methylation. The acetylation status of histones is determined by the antagonistic action of histone acetyltransferases and histone deacetylases. The largest family of histone acetyltransferases, the MYST (*M*oz, *Y*bf2/Sas3, *S*as2, *T*ip60) family is the topic of this review. As excellent recent reviews have addressed the biochemical function of MYST proteins and their roles in cell biology,(1–6) here we only briefly summarize some biochemical and cell biological properties, while focusing on the function of MYST proteins *in vivo*.

A brief overview of structure, biochemical, and cellular functions

There are five MYST histone acetyltransferases in mammals: Moz (Myst3/Kat6a), Qkf (Myst4/Morf/Kat6b), Mof (Myst1/Kat8), Tip60 (Kat5/Htatip), and Hbo1 (Myst2/Kat7). They are defined by their MYST histone acetyltransferase domain and fall into three subgroups: Moz and Qkf, Mof and Tip60, as well as Hbo1 alone, based on additional, shared protein domains (Fig. 1). The MYST domain is the catalytic domain. It contains an acetyl-coenzyme A binding domain and an unusual C2HC-type zinc finger. MYST proteins or isolated MYST histone acetyltransferase domains acetylate free histones *in vitro*.(7–11) Acetylation of histone residues is generally associated with either *de novo* histone deposition during DNA replication or with transcriptional activation.(12) Nevertheless, transcriptional repression has also been attributed to MYST histone acetyltransferases.(13–15) In addition, acetylation of non-histone targets by MYST protein has been reported in cell

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Abbreviations: AML1/Runx1, acute myeloid leukemia protein 1/runt related transcription factor 1; *at*, *Arabidopsis thaliana*; ATM, ataxia telangiectasia mutated; BCR-Abl, breakpoint cluster region/ABL1 receptor tyrosine kinase fusion (Abelson murine leukemia viral oncogene), Philadelphia chromosome translocation found in chronic myeloid leukemia, Brpf1, bromodomain and PHD finger containing protein 1; CBP, CREB binding protein; Cdt1, chromatin licensing and DNA replication factor 1; *ce*, *Caenorhabditis elegans*; Cham, chameau; c-Kit, c-kit oncogene (v-kit oncogene of the Hardy-Zuckerman-4 feline sarcoma virus), steel factor receptor; c-Mpl, c-myeloproliferative leukemia virus oncogene; *dm*, *Drosophila melanogaster*; *dr*, *Danio rerio*; Enok, enoki, mushroom; Esa1, essential Sas2-related acetyltransferase; FAB M4/5, French-American-British classification of acute leukemia M4/5; H4K16, histone 4 lysine 16; Ham1 and Ham2, histone acetyltransferase of the MYST family 1 and 2; Hbo1, histone acetyltransferase binding to Orc1; Hoxa9, homeobox protein a9; ING4 and 5, inhibitor of growth 4 and 5; Kat, lysine acetyltransferase; Mcm2, minichromosome maintenance protein 2; Mdm2, transformed mouse 3T3 cell double minute 2; MLE, maleless; MLL1, myeloid/lymphoid or mixed-lineage leukemia 1; *mm*, *Mus musculus*; Mof, males absent on the first; Moz, monocytic leukemia zinc finger protein; MSL, male-specific lethal; myc, myelocytomatosis oncogene; MYST, *M*oz, *Y*bf2/Sas3, *S*as2, *T*ip60 family of histone acetyltransferases; NCO3, nuclear receptor co-activator 3; NuA3 and 4, nucleosome acetyltransferase of H3 and 4; Orc1, origin recognition complex subunit 1; p300, E1A binding protein p300; p53, tumor suppressor protein 53; PcG, polycomb group protein; Qkf/Morf, Querkopf/Moz related factor; Rad9, DNA damage-dependent checkpoint protein Rad9; Sas2 and 3, something about silencing 2 and 3; *sc*, *Saccharomyces cerevisiae*; Sp1/Pu.1, SFFV proviral integration 1; synMUV, synthetic multivulva genes; TIF2/NCO2, transcription intermediary factor 2/nuclear receptor co-activator 2; Tip60, HIV Tat interacting protein of 60 kDa; TrxG, trithorax group proteins.

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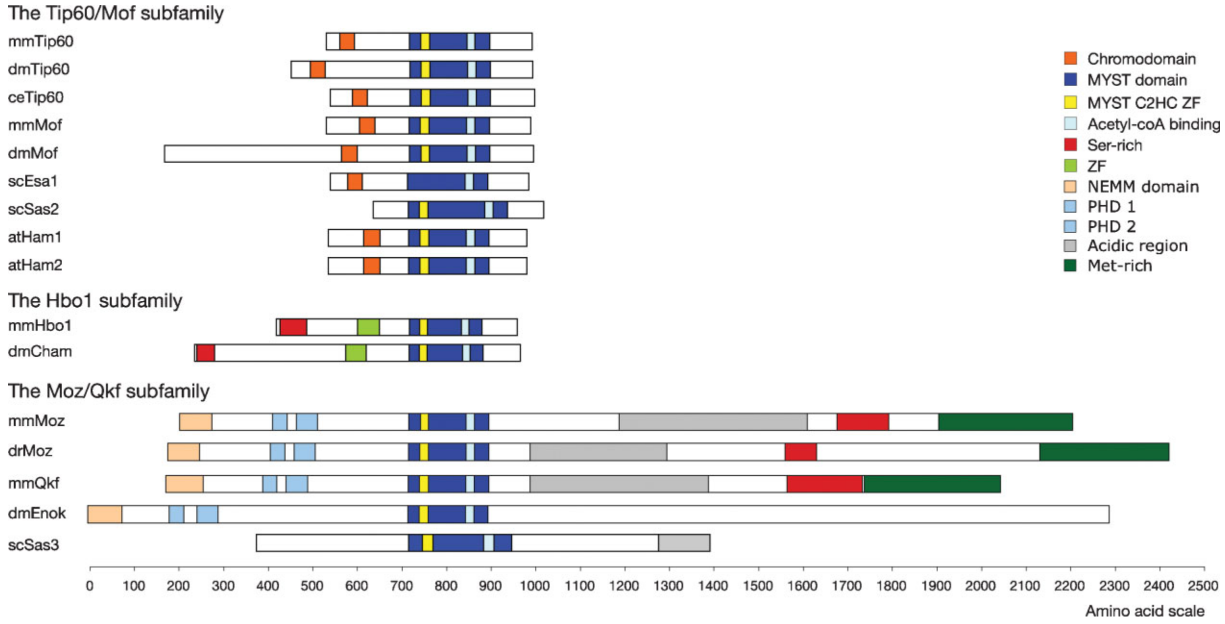

Figure 1. Members of the MYST family of histone acetyltransferases in *Mus musculus* (*mm*, representing mammals), *Drosophila melanogaster* (*dm*), *Danio rerio* (*dr*), *Caenorhabditis elegans* (*ce*), *Arabidopsis thaliana* (*at*), and *Saccharomyces cerevisiae* (*sc*) aligned according to their conserved MYST histone acetyltransferase domain (dark blue). Based on their shared protein domains MYST proteins fall into three subfamilies. Domains are colored as indicated in the legend of the graph. The C2HC zinc finger (yellow) of the MYST domain is conserved in all MYST proteins with the exception of Esa1. Moz and Qkf from animal species share two PHD fingers and a conserved N-terminal domain. The mammalian Moz and Qkf have conserved C-terminal serine- and methionine-rich domains,(11) which have been shown to interact with AML1/Runx1 and Runx2.(10,72) Mof and Tip60 share a chromodomain, which, in the case of Mof, has been shown to bind RNA.(73) Hbo1 contains an N-terminal serine-rich domain and a zinc finger domain, in addition to the zinc finger within the MYST domain.(25,33) The N-terminal portion of Hbo1 has been ascribed a transcriptional repressive function in transactivation assays *in vitro*.(33) Sas2 and Sas3 have been placed in the Tip60/Mof subfamily and the Moz/Qkf subfamily, respectively, based on their overall sequence similarity (Table 1), although they lack functional domains of their subfamilies. *D. rerio* homologs of Tip60 and Mof have not been included, as functional data for these were not available at the time of writing. MYST family proteins in other species (*Schizosaccharomyces pombe*, *Toxoplasma gondii*, *Trypanosoma brucei*, and cattle) not included in this diagram are reported elsewhere.(74–78)

culture systems. Tip60 can acetylate p53, ATM, and myc, and this can have functional consequences for the acetylated protein.(16–19) Mof can also acetylate p53(18) and can interact with ATM.(20) Therefore, the biochemical action of MYST proteins includes establishment of acetylation marks on histones and other nuclear proteins, which has consequences for transcriptional activation and transcriptional repression. As might be expected from such a broad spectrum of activities, MYST proteins are involved in a wide range of cellular processes. Nevertheless, analysis of mutants in a wide range of organisms shows that the key functions of individual MYST proteins in development are surprisingly specific. These specific functions will be discussed later.

MYST proteins occur in complex with other proteins. The yeast NuA3 and NuA4 complexes contain Sas3 and Esa1, respectively.(21,22) The mammalian complex corresponding to the yeast NuA4 complex contains Tip60.(21) MOZ and MORF were co-purified from HeLa cells in the ING5 tumor suppressor complex, while HBO1 can occur in the ING4 and the ING5 complex.(23,24) HBO1 was first identified as a protein binding to the origin of replication complex protein 1 (ORC1),(25) but does not interact with ORC1 when complexed with ING5.(23) Mof occurs in a complex first identified as the *Drosophila melanogaster* male-specific lethal complex (MSL),(26) and a human complex incorporating homologs proteins has been reported.(27) In addition, MOF can also form part of a complex containing the trithorax group protein, MLL1, in HeLa cells.(28) Interestingly, this MOF-MLL complex appears to be distinct from the MSL complex, as another male-specific lethal protein, MSL1, is not present in the MOF-MLL1 complex.(28) Mof histone acetyltransferase activity and MLL1 histone methyltransferase activity are both required for optimal transcriptional activation *in vitro*.(28)

Interestingly, not all phyla or even classes possess representatives of all MYST family subgroups (Fig. 1). While mammals and *D. melanogaster* have the three subgroups, zebrafish have representatives of the Moz/Qkf and the Mof/Tip60 subgroup, but to date no Hbo1 homolog has been described. *Saccharomyces cerevisiae* have only one representative of the Mof/Tip60 subgroup, Esa1, and possess other, comparably unrelated MYST proteins, Sas2 and Sas3, although Sas3 has an acidic region,(22) as do Moz and Qkf.(11)

Finally, *Arabidopsis thaliana* maintain only representatives of the Mof/Tip60 subgroup and no other MYST proteins.(29) These divergent complements of members of the MYST family suggest that functional diversification and specialization may have occurred in MYST proteins between the different kingdoms, phyla, and classes.

Gene expression

The expression patterns of the *Moz*, *Qkf*, *Mof*, and *Tip60* genes during mouse development and in adult tissues have been described.(11,30–32) All four MYST family members are expressed at moderate levels in all organs. However, Qkf shows dramatic up-regulation in the developing nervous system, facial structures, and limb buds,(11) while Mof and Tip60 show very high expression during spermatogenesis.(31) Expression of the *Hbo1* gene was described in adult tissues and, like other MYST genes, *Hbo1* is expressed widely in all tissues. Conflicting data suggest highest expression levels of *Hbo1* either in the ovaries(25) or in the testes.(33) The wide distribution of mRNA transcripts of all MYST family members suggests roles in a large range of cell types in various functional states including mitotic and post-mitotic cells.

Males absent on the first (Mof)

Mof is by far the most thoroughly studied member of the MYST family with respect to *in vivo* data. Although not the first MYST protein described, Mof was the first for which loss-of-function data were reported in an animal species, namely its essential role in sex chromosome dosage compensation in *Drosophila*. Mof (*m*ales absent *o*n the *f*irst) null mutation is lethal for male flies and so leads to an absence of male offspring.

Placental mammals inactivate one of the two female X chromosomes to ensure the same mRNA levels for most X chromosome-linked genes in males and females. In contrast, in *Drosophila*, gene expression from the single male X chromosome is up-regulated twofold to compensate for the number of X chromosomes. Up-regulation of gene transcription from the single male X chromosome requires the X chromosome dosage compensation complex and is vital for male survival. Mof was identified in a screen for genes required for X chromosome dosage compensation.(26) Male *mof* mutant flies with a single amino acid change in the acetyl-coenzyme A binding site die at the third instar larvae stage and fail to undergo metamorphosis. The recruitment of specific dosage compensation complex proteins, in particular maleless (MLE), to the male X chromosome is reduced and H4K16 is not hyperacetylated at the male X chromosome of *mof* mutant larvae.(26) Indeed, Mof directly acetylates H4K16 and thereby relieves chromatin-mediated repression of gene transcription.(34) H4K16 hyperacetylation is associated with hyperexpression of the single male X chromosome in flies(35) and, contrasting accordingly, the inactivated X chromosome in human cells is hypoacetylated at the same histone residue.(36)

Two groups published the consequences of loss of Mof function in mice. Both groups reported that all *Mof* null embryos, male and female, are arrested in development at the blastocyst stage and lack H4K16 acetylation.(37,38) While H4K16 acetylation is absent in *Mof* null embryos, acetylation of other histone lysine residues – H3K9, H3K14, H4K5, H4K8, and H4K12 – is normal,(38) indicating specificity of Mof for H4K16 acetylation. Lack of Mof eventually leads to apoptosis. It is important to note, however, that global and severe chromatin condensation precedes apoptotic chromatin redistribution in the *Mof* mutant embryonic nuclei. Specifically, severe chromatin condensation is seen in *Mof* mutant cells before activation of caspase 3 and before DNA fragmentation can be detected.(38) MOF is also required for H4K16 acetylation in human cells *in vitro*, suggesting that it is the principal enzyme acetylating this residue.(39)

While performing the same biochemical function of H4K16 acetylation, and presumably affecting gene transcription in both organisms (Fig. 2), Mof appears to play different roles in mammals and flies, *i.e.*, prevention of global chromatin

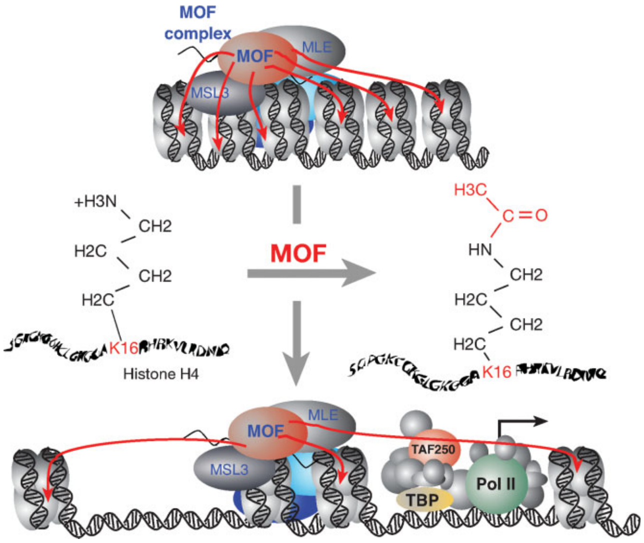

Figure 2. Schematic drawing of the most firmly established function of a MYST family member in multicellular organisms. Mof is solely responsible for H4K16 acetylation in mouse blastocysts,(37,38) which is required for the maintenance of euchromatin.(38) In the fly Mof is required for sex chromosome dosage compensation acting in the MSL complex(26) (which also contains Msl1–3, Mle, and RNA) to acetylate H4K16 and to increase gene transcription from the single male X chromosome.(34) A multiprotein complex containing homologs of the MSL complex proteins exists in human cells.(39)

condensation *versus* X chromosome dosage compensation. However, it was recently shown that mammals undergo X chromosome to autosome dosage compensation such that the single male X chromosome and the active female X chromosome are subject to a twofold up-regulation of gene expression.(40) It is therefore possible that Mof may be required for this form of dosage compensation in mammals in addition to its global role in preventing chromatin condensation. It is currently an open question as to why Mof seems to have a lesser role in acetylation on autosomes in *Drosophila* as compared to mammals.

A. thaliana has only two MYST proteins, Ham1 and Ham2. More than 90% of their amino acids are either identical or strongly similar (Table 1) and these proteins fall into the Mof/Tip60 subfamily (Fig. 1). Ham1 and Ham2 are important for gametophyte development.(29) Ham1 and Ham2 are functionally redundant, and single mutants are viable and normal under standard growth conditions. In contrast, both male and female *Ham1:Ham2* double-mutant *A. thaliana* gametophytes show abnormal development. Male double-mutant microspore mother cells and female megaspore mother cells undergo meiosis normally. Subsequently, both male and female gametophytes fail at the first post-meiotic, mitotic divisions. Fertile male pollen grains are still formed, although the lack of post-meiotic pollen mitosis I and II leads to fewer *Ham1:Ham2* double-mutant pollen grains. Failure of the first post-meiotic, mitotic divisions in the female megagametogenesis, however, results in infertile *Ham1:Ham2* double-mutant ovules.(29)

Mof mutant mouse embryos arrest in development at the blastocyst stage.(37,38) The major difference to mammals is that *A. thaliana* gametes undergo mitotic division after meiosis and before fertilization. Considering that it is these first mitotic division as *Ham1:Ham2* double-mutant micro- or megaspores that fail, *Ham1:Ham2* double-mutant *A. thaliana* arrest in development due to failure of a similar process as *Mof* mutant mouse embryos, *i.e.*, arrest of mitotic divisions after meiosis, presumably as soon as parental stores of *Mof* or *Ham1/Ham2* mRNA and protein are depleted. One would expect that Ham1 and Ham2 are required in other proliferating cells later in development, a notion supported by the fact that *Ham1* and *Ham2* are expressed in rapidly proliferating cells.(29) However, cell cycle arrest *per se* does not prove a role in cell cycle regulation, as cell cycle arrest may occur secondarily to abnormal chromatin condensation and generalized failure of gene transcription.

A close relative of Mof in *S. cerevisiae*, Esa1 was reported to be required for cell cycle progression.(41,42) *Esa1* mutant yeast germinate, and divide three to five times before failing to distribute their DNA between daughter cells. In addition, *esa1* mutant yeast exhibit abnormal distribution of electron-dense

Table 1. Amino acid sequence identity or strong similarity in the MYST domains[a] and the full-length sequence

Overall\MYST	mmTip60	dmTip60	ceTip60	mmMof	dmMof	scEsa1	scSas2	atHam1	atHam2	mmHbo1	dmCham	mmMoz	drMoz	mmQkf	dmEnok	scSas3
mmTip60		**94.5%**[b]	**89.5%**[c]	80.1%	77.9%	81.2%	61.0%	81.8%	82.3%	83.4%	79.5%	78.5%	77.9%	78.5%	79.0%	74.7%
dmTip60	**66.2%**[b]		**89.5%**[c]	81.2%	79.0%	81.8%	63.2%	**84.0%**[b]	**84.0%**[b]	82.9%	79.5%	80.7%	79.6%	80.7%	80.1%	75.3%
ceTip60	**65.4%**[b]	58.6%		78.5%	77.9%	**83.4%**[b]	62.6%	79.0%	79.0%	82.3%	77.8%	81.2%	79.6%	79.0%	79.6%	74.2%
mmMof	55.7%	51.5%	57.4%		**87.8%**[b]	78.5%	63.2%	82.3%	81.8%	82.3%	78.4%	80.7%	81.2%	81.8%	81.8%	73.7%
dmMof	35.6%	37.4%	32.5%	**43.5%**[b]		76.8%	**64.8%**[b]	80.7%	81.2%	81.8%	75.7%	81.8%	83.4%	80.7%	82.9%	73.7%
scEsa1	61.0%	53.5%	**65.0%**[b]	58.6%	33.4%		56.6%	80.1%	80.7%	79.6%	75.1%	75.7%	76.3%	76.8%	80.1%	76.3%
scSas2	40.3%	34.7%	40.7%	40.4%	23.6%	41.9%		60.4%	60.4%	62.6%	61.8%	61.5%	61.5%	62.6%	61.5%	55.0%
atHam1	57.4%	53.4%	57.6%	**67.3%**[b]	37.8%	58.2%	42.9%		**98.3%**[d]	82.3%	75.7%	79.6%	80.7%	80.1%	81.2%	72.1%
atHam2	57.7%	53.1%	57.0%	**67.1%**[b]	37.9%	60.0%	**43.3%**[b]	**93.5%**[d]		83.4%	75.7%	80.1%	80.7%	80.7%	82.3%	73.2%
mmHbo1	48.1%	48.2%	47.6%	49.6%	39.6%	48.2%	33.3%	49.3%	**50.9%**[b]		**85.6%**[b]	85.6%	86.2%	85.1%	**89.5%**[b]	76.3%
dmCham	34.4%	37.3%	33.4%	37.9%	41.3%	33.2%	24.2%	36.4%	35.5%	**50.4%**[b]		81.6%	79.5%	82.2%	82.7%	73.2%
mmMoz	14.5%	14.9%	14.2%	14.6%	17.4%	13.2%	9.9%	13.9%	14.5%	16.8%	18.9%		**97.6%**[b]	**96.7%**[d]	**90.6%**[c]	74.2%
drMoz	12.8%	13.1%	12.4%	13.2%	15.8%	12.0%	8.6%	11.9%	13.0%	15.4%	15.3%	**63.5%**[b]		**95.5%**[b]	**90.6%**[c]	74.7%
mmQkf	14.5%	16.4%	14.8%	16.2%	18.4%	14.1%	10.2%	13.7%	15.2%	17.7%	19.4%	**65.5%**[d]	**56.5%**[b]		**90.6%**[c]	74.7%
dmEnok	11.6%	12.9%	11.9%	12.7%	17.3%	11.8%	8.5%	12.1%	11.9%	16.3%	18.7%	**38.1%**[c]	36.2%	**38.1%**[c]		**78.4%**[b]
scSas3	32.1%	**33.9%**[b]	32.6%	31.1%	29.4%	33.0%	23.4%	30.8%	31.8%	33.4%	28.7%	21.3%	20.7%	22.5%	19.2%	

[a] Comparison of the core, most conserved 180 amino acids of the MYST domain beginning and ending with a conserved residue, *i.e.*, excluding the first eight and the last two amino acids of the MYST domain shown previously.(11)

[b] Indicates best match between species.

[c] Indicates ambiguous match between species.

[d] Indicates match within species better than between species.

Two letters before protein abbreviate species nomenclature as in the text. Note that best matches are not reciprocal in all cases such that two "best matches" referring to two different proteins can occur per column or row. For example, the overall sequence of mmMof is most closely related to atHam1 (67.3%). However, atHam1 is much more closely related to atHam2 (93.5%). Conversely, the overall sequence of dmEnok is equally distantly related to mmMoz and mmQkf (both 38.1%) and its MYST domain is equally closely related to mmMoz, drMoz, and mmQkf (90.6%). Similarities between protein sequences were analyzed using CLUSTAL W.(79) *D. rerio* homologs of Tip60 and Mof have not been included, as functional data were not available for these at the time of writing.

Bold used highlight the pairs with highest sequence similarities, which are further delineated by superscripts b,c,d.

nuclear material.(42) Atypically, the arrest in cell cycle is followed by cell death in *esa1* mutants, whereas classical cell cycle mutants are viable. Although this might suggest that cell cycle arrest in the *esa1* mutant yeast may be a consequence of abnormal chromatin structure, as seen in mouse preimplantation embryos, the cell cycle arrest of *esa1* mutant yeast was rescued by mutation of *rad9*.(42) Rad9 is a cell cycle checkpoint protein involved in sensing DNA damage. So it would appear that Esa1 plays a role in maintaining the integrity of the DNA, rather than open chromatin structure and high-level transcriptional activity, the roles attributed to mouse and *D. melanogaster* Mof.

Maintaining an open chromatin structure, *i.e.*, protecting euchromatin from condensation and from the spreading of heterochromatin, was attributed to another *S. cerevisiae* MYST protein, Sas2.(43,44) Two groups found that Sas2 histone acetylation of H4K16 opposed by Sir2 deacetylation of H4K16 at the euchromatin/heterochromatin interface maintains the boundary between regions of transcriptionally active and silent telomeric chromatin. While previous, conflicting evidence suggested that Sas2 might either promote or inhibit transcriptional silencing,(15,45) genome-wide analysis of wild-type *versus sas2* mutant yeast supports a role of Sas2 in the maintenance of transcriptionally active euchromatin.(43)

The activity of Sas2 matches the function of mammalian Mof with respect to the specific histone lysine residue (H4K16) and consequences for the chromatin state (maintenance of euchromatin). While it appears that the effects of *sas2* mutation in yeast are quite specific to the euchromatin/heterochromatin boundary,(43) and the effects of mutating *mof* in flies are certainly most pronounced at and most relevant to the male X chromosome,(26,34) it is yet unknown if mutation of *Mof* in mammals affects all areas of the genome equally.

Concluding from reports on Mof in flies and mice *versus* Esa1 and Sas2 in yeast, the functional comparison of animal and yeast MYST family members does not yield pairs of proteins with greatest sequence similarity and equivalent function. Rather functional diversification and also convergence seems to have occurred during evolution.

HIV Tat interacting protein of 60 kDa (Tip60)

In the previous section, we considered the possibility that yeast Esa1, although closely related to Mof in amino acid sequence and protein domain structure, may not perform the equivalent function in euchromatin maintenance/transcription, but rather have a role in maintaining DNA integrity and cell cycle progression. The closest mammalian relative of Esa1, Tip60 has the same protein domain structure as Mof and Esa1 (Fig. 1 and Table 1). Tip60 occurs in a complex homologous to the yeast NuA4 complex, which contains Esa1 in yeast.(21) Like Esa1, Tip60 has been reported to play essential roles in cell cycle progression *in vitro*. Furthermore, both Esa1(46) and Tip60(47,48) play essential roles in DNA double-strand break repair. Tip60 may therefore be functionally more similar to Esa1 than to Mof.

While it was reported that *Tip60* mutant mouse embryos die before implantation,(49) events leading to the death, the mode of death of *Tip60* mutant mouse embryos, and their histone acetylation status have not been reported to date. Depletion of Tip60 by RNAi in *D. melanogaster* leads to developmental lethality before or at the early pupal stage,(50) but neither cellular events surrounding death nor histone acetylation status were reported.

Interestingly, rather than leading to developmental arrest or lethality, RNAi knockdown of *Tip60* in *Caenorhabditis elegans* leads to a recruitment of six instead of three cells into the vulval cell fate, the multivulva (MUV) phenotype.(51) In this context Tip60, in complex with homologs of the mammalian Tip60 complex, exhibits functional redundancy with two other groups of genes, known as synthetic multivulva A and B genes (synMUV). Therefore, the genes encoding proteins of the Tip60 complex were termed class C synMUV genes. As synMUV A and B counteract EGF to Ras to MAPK signaling and the Tip60 complex is a chromatin-modifying complex, it was suggested that chromatin modification may be a general mechanism to limit signaling during development. However, global histone acetylation was found unchanged in the *Tip60*-depleted *C. elegans* when compared to wild type.(51) The normal fate of the three supernumerary vulva cells in the *Tip60* mutants is to divide once more and to generate daughter cells that fuse with the syncytial hypodermis. Therefore, Tip60 depletion in *C. elegans* results in premature cell cycle exit and differentiation and, as such, Tip60 may potentially control cell cycle exit and differentiation in this organism, *albeit* restricted to one particular cell type.

Several non-histone acetylation targets have been reported for Tip60. Apart from p53 and ATM in the context of cell cycle arrest and apoptosis in a variety of human cancer cell lines,(17–19) Tip60 can acetylate c-Myc. This results in increased c-Myc protein stability in transfected H1299 human lung carcinoma cells.(16) Myc recruits the Tip60 complex to the chromatin in Rat1 wild-type cells, but not in Rat1 *Myc* mutant cells.(52) However, expression of a mutant form of Tip60 did not interfere with the activation of Myc target genes.(52) Therefore, it remains unclear if Tip60 enhances Myc function (and proliferation). Data on *C. elegans Tip60* mutants suggest that Tip60 might prevent cell cycle exit and differentiation, a prerequisite for continued proliferation. Interestingly, an RNAi screen in mouse embryonic stem cells revealed that Tip60 is required for pluripotency, and genome-wide expression analysis of Tip60-depleted ES cells suggests that Tip60 represses a large number of genes that are expressed during differentiation.(53)

Overall, the role of Tip60 *in vivo* remains unclear. Differences exist between mouse, *D. melanogaster* and *C. elegans* with respect to onset and type of Tip60 loss-of-function abnormalities. However, in particular the difference in type of abnormality, *i.e.*, lethality in mouse and fly *versus* cell fate mis-specification in the worm, could either be the result of functional diversification or due to the experimental approach, *i.e.*, null mutation *versus* knockdown.

Histone acetyltransferase binding to ORC1 (Hbo1)

Unlike other mammalian MYST family members, Hbo1 appears to function predominantly in transcriptional repression. Null mutation of the *Hbo1* homolog in *D. melanogaster, chameau*, leads to lethality at the pupal stage.(14) Interestingly, informative effects on epigenetic gene repression were observed in *chameau* heterozygous flies. Haploinsufficiency for *chameau* leads to defects in position effect variegation, in polycomb group protein (PcG) mediated repression of homeotic genes and consequent homeotic transformation in thoracic and abdominal segments. Compound heterozygous flies for *chameau* and the PcG genes *polycomb*, *polyhomeotic*, or a mutation in a polycomb response element, *Mcp*, showed more pronounced homeotic transformation than heterozygotes of the PcG genes alone.(14) The genetic interactions show that chameau cooperates with PcG proteins to repress *Hox* genes and to specify body segments identity. Similarly, Hbo1 was shown to interact with the androgen receptor to repress androgen receptor-mediated reporter gene transcription when overexpressed in CV-1 and PC3 cells.(33) Interestingly, in another developmental process, chameau cooperates with the JNK signaling to promote thorax closure in the adult form of *D. melanogaster*.(54) In HEK293 it was shown that chameau can cooperate with DFos and DJun to promote transcription of a gene from a AP1 promoter and concomitant with increasing H4K16 acetylation.(54)

Hbo1 is not the only MYST histone acetyltransferase implicated in transcriptional repression. The something about silencing proteins, Sas2 and Sas3, were originally identified in *S. cerevisiae* as two proteins that enhanced Sir1-mediated epigenetic gene silencing.(15) The fact that chameau expression can rescue the subtelomeric reporter transgene silencing defect in *sas2* mutants(14) needs to be considered in the light of conflicting results on Sas2 function. Sas2 is required for subtelomeric reporter transgene silencing,(15) but also for transcriptional activity of transgenes integrated into rDNA,(45) for transcriptional activation of a mutated HMRE silent mating type locus(15) and for protection of euchromatin from heterochromatin spreading.(43,44) As functions both promoting and counteracting silencing are possible for Sas2, it is possible that histone acetylation by Sas2 could promote expression of genes coding for silencing proteins or Sas2 might acetylate a silencing protein that is only active in its acetylated form.

On the surface, chameau function in flies is at odds with a previous report of Hbo1 function in cell culture. HBO1 occurs as a component of a multiprotein complex with histone H3 and H4 acetyltransferase activity in 293 cells.(25) A portion of HBO1 associates with the origin of DNA replication complex protein ORC1,(25) the minichromosome maintenance protein MCM2,(55) and the replication licensing factor CDT1 in proliferating cells,(56) suggesting that HBO1 might perform a role in DNA replication. Moreover, RNAi depletion of HBO1 in 293T cells resulted in an accumulation of cells in S phase of the cell cycle.(23) Importantly, a genome-wide increase in histone acetylation stimulates replication independently of transcription in *D. melanogaster* follicle cells.(57) However, the survival of *chameau* null flies to the pupal stage suggests that cell proliferation, and so DNA replication, can occur in the absence of chameau, although the condition of the *chameau* null pupa has not been described. Furthermore, some Orc proteins, in addition to their function in DNA replication, have roles in transcriptional repression in position effect variegation and heterochromatin formation in *D. melanogaster*(58) as well as roles in gene silencing in *S. cerevisiae*.(59–61) Therefore, an interaction of HBO1 with ORC proteins neither conclusively proves a role in DNA replication nor excludes a role in other cellular processes.

The data obtained on Hbo1 function thus allow a number of hypotheses: (1) Hbo1 may acetylate histones or non-histone proteins to promote DNA replication; (2) Hbo1 may establish specific histone acetylation marks required for transcriptional repression, *e.g.*, for the recruitment of silencing proteins; (3) Hbo1 may acetylate a transcriptional repressor that is only active in its acetylated form; and/or (4) Hbo1 may be critically important for the expression of a transcriptional repressor. As the *Hbo1* gene is expressed both in tissues with proliferating cells, such as testes, and in tissues almost exclusively composed of post-mitotic cells, such as the brain, it is likely that Hbo1 has roles other than, or in addition to, promoting DNA replication.

Querkopf/Moz related factor (Qkf/Morf)

A mouse *Qkf* gene trap allele, Qkf^{gt}, was generated in a genetic screen for genes required for embryonic development.(11,62) $Qkf^{gt/gt}$ mutants are of a normal size at birth, but then fail to thrive.(11) $Qkf^{gt/gt}$ mutants that survive to weaning age (less than 50% on an inbred *129Sv/Pas* background) are smaller than littermate controls, have craniofacial abnormalities, skeletal abnormalities, and a disproportional reduction in the size of the cerebral cortex even when the difference in body size is taken into consideration. The developing $Qkf^{gt/gt}$ mutant forebrain at embryonic day 11.5 contains fewer cerebrocortical progenitor cells; the cerebral cortex primordium, the cortical plate, contains fewer neurons and is reduced in size at embryonic days 13.5, 15.5, and 17.5.(11) As DNA synthesis does not appear to be disrupted,(11) the reduction in neural progenitors and neurons suggests that *Qkf*$^{gt/gt}$ mutant neural progenitor cells exit the cell cycle prematurely.

The *Qkf* mutant neurogenesis defect extends into adult life, when the subventricular neurogenic region has a reduced number of neural stem cells(63,64) and produces fewer migrating neuroblasts as well as fewer olfactory interneurons.(64) Consequently, the *Qkf* mutant olfactory bulbs, unlike wild-type olfactory bulbs, fail to grow in adult life.(64) Moreover, *Qkf* mutant neural stem and progenitor cells fail to undergo self-renewal and give rise to fewer neurons *in vitro*. Conversely, overexpression of *Qkf* leads to increased numbers of differentiating neurons.(64) Taken together, Qkf is required for the maintenance of undifferentiated neuronal progenitors and for adult neural stem cell self-renewal and neuronal differentiation.

Strikingly, the *D. melanogaster* homolog of Moz and Qkf, Enok, has a very similar function in flies. Enok is essential for mushroom body development.(65) The mushroom bodies are the sites of olfactory learning and memory and in this function equivalent to the mammalian brain. Both an *enok* null allele and a point mutation in the zinc finger of the MYST histone acetyltransferase domain cause an arrest in neuroblast proliferation.(65) *Enok* mutation also results in a slow, but steady decline of egg production over time. However, no difference was observed in the proliferation of wing disk cells.(65) Defects in *enok* mutant neuron and egg production were interpreted as proliferation defects. It is possible that the *enok* mutant neuroblasts and germ cells differentiated prematurely.

Therefore, the emerging function of Qkf/Morf is a requirement in neural stem cell/neural progenitor self-renewal with an additional role in some other cell types such as osteoblasts and germ cells. It is important to note, however, that the *Qkf* gene, in addition to being strongly expressed in the developing cerebral cortex and in adult neural stem cells, is also expressed in post-mitotic cells such as neurons,(11,64) where it is presumably involved in cellular processes other than self-renewal.

Monocytic leukemia zinc finger protein (Moz)

The loss of function of *Moz* in mice was reported by two groups, who generated different *Moz* mutant alleles with similar consequences for the hematopoietic system.(30,66) In one case the *Moz* mutant mice develop to term(30) and in the other case to embryonic day 15.5.(66) Both alleles cause semi-dominant defects in the hematopoietic stem cell compartment. Hematopoietic stem cells, as assayed by competitive reconstitution assay of lethally irradiated mice, are absent from *Moz* homozygous mutant fetal livers and reduced in number in *Moz* heterozygous mutant fetal livers, showing that even a gradual reduction in Moz levels impairs hematopoietic stem cell development. *Moz* homozygous mutant hematopoietic progenitor cells, as assayed by flow cytometry and colony formation *in vitro*, are severely reduced in number. However, progenitor lineage commitment and differentiation are not affected, as they form colonies of all cell types(30,66) and produce all peripheral blood cell types at birth.(30) Both groups observed a partial block in the late stage erythroblasts maturation. In addition, dysplasia and hypocellularity of the fetal liver, thymus, spleen, and bone marrow were observed in *Moz* mutant fetuses.(30)

Different results were reported by two groups with respect to the granulocyte and monocyte lineage. While in one case no difference was observed between mutants and controls in the peripheral blood granulocytes and monocytes at birth,(30) in the other case some animals appeared to have increased numbers of *Moz* mutant fetal liver granulocytes and monocytes.(66) However, data compiled from nine *Moz* mutants and six controls showed no significant difference in *Moz* mutant fetal liver granulocytes and monocytes.(66)

Based on similarities to the loss-of-function phenotype of *Moz* mutants with those mutants of the Runt family transcription factor gene *Aml1/Runx1* and the Ets family transcription factor gene *Pu.1* and on co-immunoprecipitation and transcriptional activation assays, it was suggested that Moz may affect the function of both Pu.1 and Aml1 in hematopoiesis and leukemia.(10,66) Furthermore, since *Moz* deficiency leads to a reduction in the expression of the homeobox transcription factor gene *Hoxa9*, the thrombopoietin receptor gene *c-Mpl*, and the stem cell factor receptor gene *c-Kit*, it was proposed that these are target loci of Moz function.(66)

As *Moz* mutant pups develop to a normal size with normal-sized organs apart from hematopoietic organs,(30) the requirement for Moz does not seem to extend to many other stem cell populations, but rather seems to be specific to hematopoietic stem cells. The histone acetyltransferase activity is required for Moz to support hematopoietic stem cell function.(67)

Interestingly, mammalian Moz and its homolog in *Danio rerio* zMoz appear to have diverged in their expression domains. While the mouse *Moz* gene is expressed throughout the developing embryo and in most adult organs,(30) *zMoz* expression is restricted to the zebrafish head.(68) Consequently, morphological abnormalities are likewise restricted to the head region.(68,69) Surprisingly, there does not appear to be another homolog for Moz in zebrafish to perform a potential function of Moz in the trunk region. Zebrafish homozygous for ENU mutations in the *zMoz* gene exhibit pharyngeal arch segment identity defects extending to skeletal and nervous system elements of the head.(68,69) *Hox* genes are known to confer body segment identity. Commensurate with the observed phenotypic abnormalities, expression levels of *Hox* genes in the head region, specifically levels of *hoxb1a*, *hoxb2a*, *hoxb2b*, *hoxb3a*, and *hoxb4a* mRNA (but not *hoxa1a* or *hox5* to *6*) are reduced in *zMoz* mutants.(68) Morpholino-mediated depletion of both *zMoz* and the *zBrpf1* gene, which encodes the bromodomain and PHD finger protein 1, in zebrafish showed that both zMoz and zBrpf1 are required to activate *Hox* gene expression.(70) Furthermore, zMoz and zBrpf1 co-immunoprecipitated when overexpressed in HEK293 cells.(70) A potential role of Moz in zebrafish hematopoiesis was not reported. Interestingly, however, overexpression of the human MOZ-TIF2 fusion protein, the product of translocations leading to acute myeloid leukemia, under the control of the *sp1 (Pu.1)* promoter induces acute myeloid leukemia in the fish.(71)

Thus, the function of mammalian and zebrafish *Moz* appears to be conserved with respect to regulation of *Hox* gene expression and the potential of inducing leukemic stem cells, as a fusion protein with TIF2 (see Information box). In contrast, the extent of *Moz* gene expression and potential functions outside of the head region do not appear to be conserved between fish and mouse.

Commonalities in the function of Moz and the closely related Qkf/Morf protein in diverse stem cell populations are striking. Hematopoietic stem cells fail to develop in *Moz* mutant mice and neural stem cells have a self-renewal defect in *Qkf* mutants. In contrast, proliferation of hematopoietic progenitor cells and many other cell types appear normal in the *Moz* and Qkf mutant mice, which are of normal size at birth. It appears, therefore, that Moz and Qkf are required individually in specific stem cell populations, but not for proliferation in general. Considering the high degree of sequence similarity of Moz and Qkf (>90% in three of four functional domains) and their widespread expression, it is nevertheless possible that proliferating cells require one of the pair of Moz and Qkf (Figs. 3 and 4).

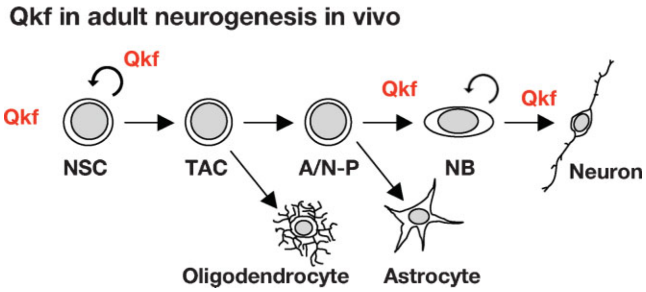

Figure 3. Lack of Qkf causes an adult neurogenesis defect. The *Qkf* mutant subventricular neurogenic region has a reduced number of neural stem cells,(63,64) produces fewer migrating neuroblasts and fewer olfactory interneurons.(64) Qkf is required during development for the maintenance of neuronal progenitor cells and for adult neural stem cell self-renewal and neuronal differentiation.

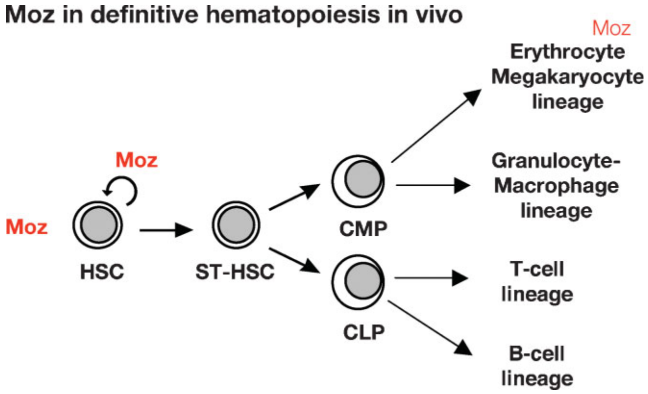

Figure 4. The loss of function of *Moz* causes defects in the hematopoietic stem cell compartment. Hematopoietic stem cells are absent from *Moz* homozygous mutant fetal livers and reduced in number in *Moz* heterozygous mutant fetal livers, showing that even a gradual reduction in Moz levels impairs hematopoietic stem cell development.(30,66) The action of Moz is almost entirely restricted to the stem cell compartment, which has strong parallels to the function of Qkf in neurogenesis. However, Qkf also has a role in the differentiation of neurons.

Conclusion and outlook

MYST family histone acetyltransferases appear to have diverse functions. The roles most securely established *in vivo* are: (a) the global maintenance of transcriptionally active chromatin *via* H4K16 acetylation by Mof in mice and flies (Fig. 2) as well as Sas2 in yeast; (b) the regulation of self-renewal in hematopoietic stem cells by Moz, in adult neural stem cells by Qkf, in neural precursor cells by Qkf in mice, and Enok in flies; and (c) transcriptional repression of specific target loci by Hbo1 in flies (Table 2). Although we know that zygotic production of Tip60 becomes critical at implantation, the cellular mechanisms requiring Tip60 *in vivo* have not been described.

Loss-of-function studies have provided an insight into the diverse cellular functions of MYST proteins, but many questions remain unanswered. What are the DNA-binding proteins that need this class of histone acetyltransferases? Are they used promiscuously or are certain MYST proteins always acting in conjunction with specific proteins? Mof acetylates H4K16, but which lysines are acetylated by the other MYST family members *in vivo*? Are they specific like Mof or do they act in a context-dependent manner? And perhaps most importantly, how does lysine acetylation regulate gene expression and chromatin structure?

In the next few years we expect that many of these questions will be answered and that MYST proteins will emerge as one of the corner stones regulating chromatin state and gene expression during development, in health and disease.

Table 2. Summary of the loss-of-function phenotypes of MYST family members *in vivo*

MYST	Species	Loss-of-function phenotype	References
Mof	*dm*	Male-specific lethality, mutants fail to up-regulate expression from the single male X chromosome, failure to acetylate H4K16	Hilfiker *et al*,(26) Akhtar and Becker(34)
Mof	*mm*	Male and female mutant embryos die at the blastocysts stage; failure to acetylate H4K16, while H4K5,8,12 and H3K9,14 acetylation is normal; global chromatin condensation preceding apoptosis	Gupta *et al.*,(37) Thomas *et al.*(38)
Tip60	*mm*	Pre-implantation lethality	Gorrini *et al.*(49)
Tip60	*dm*	RNAi KD is lethal before the early pupa stage	Zhu *et al.*(50)
Tip60	*ce*	RNAi KD leads to recruitment of additional cells to a vulva cell fate	Ceol and Horvitz(51)
Esa1	*sc*	Mutants fail to divide more than five times after germination, failure to distribute DNA between daughter cells during cell division	Smith *et al.*,(41) Clarke *et al.*(42)
Sas2	*sc*	Sas2 is required for H4K16 acetylation and to prevent heterochromatin spreading	Suka *et al.*,(44) Kimura *et al.*(43)
Ham1 Ham2	*at*	Single KOs viable; Ham1 and Ham2 double KO fail at one of the first post-meiotic cell divisions during gametogenesis	Latrasse *et al.*(29)
Cham	*dm*	Lethality at the pupal stage; when *chameau* heterozygosity is combined with *PcG* mutant alleles, failure to repress homeotic genes and homeotic transformation	Grienenberger *et al*(14)
Moz	*mm*	Failure of fetal liver hematopoietic stem cells to reconstitute a lethally irradiated host; reduced number of hematopoietic progenitor cells; partial block in the late stage erythroblasts maturation; reduced expression of *Hoxa9*, *c-Mpl*, and *c-Kit*	Thomas *et al.*,(30) Katsumoto *et al.*(66)
Moz	*dr*	ENU-induced truncation mutation: Failure to specify pharyngeal arch segments correctly; reduced expression of specific *Hox* genes	Miller *et al*,(68) Crump *et al.*(69)
Qkf	*mm*	Gene trap allele producing 5% normal mRNA: reduced numbers of embryonic neural precursors; skeletal abnormalities; failure to thrive in the post-natal period; up to 80% deaths between birth and weaning; reduction in adult neural stem cells; adult neural stem cell self-renewal defect; and neuronal differentiation defect	Thomas *et al.*,(11) Merson *et al.*(64)
Enok	*dm*	Arrest in neuroblast proliferation in mushroom bodies	Scott *et al.*(65)
Sas3	*sc*	*Sas3* mutant viable, but lethal as double mutant with *gcn5*: failure to acetylate H3, cell cycle arrest in G2/M phase	Howe *et al.*(80)

KD, knockdown; KO, knockout.

Information box

The MYST family of histone acetyltransferases in human disease processes

The roles of MYST proteins in human disease, in particular their link to cancer, have been reviewed recently.(2)

Moz was identified in recurrent t(8:16)(p11;p13) translocation leading to FAB M4/5 leukemia,(81) a particularly aggressive form of leukemia with a median survival time of only 4.7 months after diagnosis.(82) Further translocations implicate MOZ in leukemia and myelodysplastic syndrome.(83–87) The products of these translocations consist of the N-terminal one-half to two-thirds of the MOZ protein, including its MYST histone acetyltransferase domain, fused to a number of different fusion partners including CBP, p300, TIF2/NCO2, and NCO3. The MOZ-TIF2 fusion protein deserves particular attention, as its overexpression in common myeloid or granulocyte-monocyte progenitor cells confers leukemic stem cell properties to the progenitor cells such that they generate serially transplantable leukemia.(88,89) In contrast, another well-known oncogene, BCR-Abl is unable to convert common myeloid or granulocyte-monocyte progenitor into leukemic stem cells.(88)

Translocations affecting **Morf** (Qkf) lead to leiomyomata, benign tumors of the uterus that affect up to 77% of women.(90) Translocations fusing Morf to CBP cause aggressive forms of childhood acute myeloid leukemia(91) and adult acute myeloid leukemia.(92,93)

Tip60 was first identified as a protein interacting with the human immunodeficiency virus tat protein (HIV-1 Tat).(94) HIV-1 Tat targets Tip60 to ubiquitination and degradation to impair Tip60-dependent apoptotic response to DNA damage.(95)

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References

1. **Yang, X. J. and Ullah, M.,** MOZ and MORF, two large MYSTic HATs in normal and cancer stem cells. *Oncogene* 2007. **26**: 5408–5419.
2. **Avvakumov, N. and Cote, J.,** The MYST family of histone acetyltransferases and their intimate links to cancer. *Oncogene* 2007. **26**: 5395–5407.
3. **Lafon, A., Chang, C. S., Scott, E. M., Jacobson, S. J. and Pillus, L.,** MYST opportunities for growth control: yeast genes illuminate human cancer gene functions. *Oncogene* 2007. **26**: 5373–5384.
4. **Pillus, L.,** MYSTs mark chromatin for chromosomal functions. *Curr Opin Cell Biol* 2008. **20**: 326–333.
5. **Thomas, T. and Voss, A. K.,** The diverse biological roles of MYST histone acetyltransferase family proteins. *Cell Cycle* 2007. **6**: 696–704.
6. **Rea, S., Xouri, G. and Akhtar, A.,** Males absent on the first (MOF): from flies to humans. *Oncogene* 2007. **26**: 5385–5394.
7. **Yamamoto, T. and Horikoshi, M.,** Novel substrate specificity of the histone acetyltransferase activity of HIV-1-Tat interactive protein Tip60. *J Biol Chem* 1997. **272**: 30595–30598.
8. **Champagne, N., Bertos, N. R., Pelletier, N., Wang, A. H., Vezmar, M.,** *et al*. Identification of a human histone acetyltransferase related to monocytic leukemia zinc finger protein. *J Biol Chem* 1999. **274**: 28528–28536.
9. **Champagne, N., Pelletier, N. and Yang, X. J.,** The monocytic leukemia zinc finger protein MOZ is a histone acetyltransferase. *Oncogene* 2001. **20**: 404–409.
10. **Kitabayashi, I., Aikawa, Y., Nguyen, L. A., Yokoyama, A. and Ohki, M.,** Activation of AML1-mediated transcription by MOZ and inhibition by the MOZ-CBP fusion protein. *EMBO J* 2001. **20**: 7184–7196.
11. **Thomas, T., Voss, A. K., Chowdhury, K. and Gruss, P.,** Querkopf, a MYST family histone acetyltransferase, is required for normal cerebral cortex development. *Development* 2000. **127**: 2537–2548.
12. **Strahl, B. D. and Allis, C. D.,** The language of covalent histone modifications. *Nature* 2000. **403**: 41–45.
13. **Clarke, A. S., Samal, E. and Pillus, L.,** Distinct roles for the essential MYST family HAT Esa1p in transcriptional silencing. *Mol Biol Cell* 2006. **17**: 1744–1757.
14. **Grienenberger, A., Miotto, B., Sagnier, T., Cavalli, G., Schramke, V.,** *et al*. The MYST domain acetyltransferase Chameau functions in epigenetic mechanisms of transcriptional repression. *Curr Biol* 2002. **12**: 762–766.
15. **Reifsnyder, C., Lowell, J., Clarke, A. and Pillus, L.,** Yeast SAS silencing genes and human genes associated with AML and HIV-1 Tat interactions are homologous with acetyltransferases. *Nat Genet* 1996. **14**: 42–49.
16. **Patel, J. H., Du, Y., Ard, P. G., Phillips, C., Carella, B.,** *et al*. The c-MYC oncoprotein is a substrate of the acetyltransferases hGCN5/PCAF and TIP60. *Mol Cell Biol* 2004. **24**: 10826–10834.
17. **Sun, Y., Jiang, X., Chen, S., Fernandes, N. and Price, B. D.,** A role for the Tip60 histone acetyltransferase in the acetylation and activation of ATM. *Proc Natl Acad Sci USA* 2005. **102**: 13182–13187.
18. **Sykes, S. M., Mellert, H. S., Holbert, M. A., Li, K., Marmorstein, R.,** *et al*. Acetylation of the p53 DNA-binding domain regulates apoptosis induction. *Mol Cell* 2006. **24**: 841–851.
19. **Tang, Y., Luo, J., Zhang, W. and Gu, W.,** Tip60-dependent acetylation of p53 modulates the decision between cell-cycle arrest and apoptosis. *Mol Cell* 2006. **24**: 827–839.
20. **Gupta, A., Sharma, G. G., Young, C. S., Agarwal, M., Smith, E. R.,** *et al*. Involvement of human MOF in ATM function. *Mol Cell Biol* 2005. **25**: 5292–5305.
21. **Doyon, Y., Selleck, W., Lane, W. S., Tan, S. and Cote, J.,** Structural and functional conservation of the NuA4 histone acetyltransferase complex from yeast to humans. *Mol Cell Biol* 2004. **24**: 1884–1896.
22. **John, S., Howe, L., Tafrov, S. T., Grant, P. A., Sternglanz, R. and Workman, J. L.,** The something about silencing protein, Sas3, is the catalytic subunit of NuA3, a yTAF(II)30-containing HAT complex that interacts with the Spt16 subunit of the yeast CP (Cdc68/Pob3)-FACT complex. *Genes Dev* 2000. **14**: 1196–1208.
23. **Doyon, Y., Cayrou, C., Ullah, M., Landry, A. J., Cote, V.,** *et al*. ING tumor suppressor proteins are critical regulators of chromatin acetylation required for genome expression and perpetuation. *Mol Cell* 2006. **21**: 51–64.
24. **Ullah, M., Pelletier, N., Xiao, L., Zhao, S. P., Wang, K.,** *et al*. Molecular architecture of quartet MOZ/MORF histone acetyltransferase complexes. *Mol Cell Biol* 2008. **28**: 6828–6843.
25. **Iizuka, M. and Stillman, B.,** Histone acetyltransferase HBO1 interacts with the ORC1 subunit of the human initiator protein. *J Biol Chem* 1999. **274**: 23027–23034.
26. **Hilfiker, A., Hilfiker-Kleiner, D., Pannuti, A. and Lucchesi, J. C.,** Mof, a putative acetyl transferase gene related to the Tip60 and MOZ human genes and to the SAS genes of yeast, is required for dosage compensation in *Drosophila*. *EMBO J* 1997. **16**: 2054–2060.
27. **Smith, E. R., Cayrou, C., Huang, R., Lane, W. S., Cote, J. and Lucchesi, J. C.,** A human protein complex homologous to the *Drosophila* MSL complex is responsible for the majority of histone H4 acetylation at lysine 16. *Mol Cell Biol* 2005. **25**: 9175–9188.
28. **Dou, Y., Milne, T. A., Tackett, A. J., Smith, E. R., Fukuda, A.,** *et al*. Physical association and coordinate function of the H3 K4 methyltransferase MLL1 and the H4 K16 acetyltransferase MOF. *Cell* 2005. **121**: 873–885.
29. **Latrasse, D., Benhamed, M., Henry, Y., Domenichini, S., Kim, W.,** *et al*. The MYST histone acetyltransferases are essential for gametophyte development in *Arabidopsis*. *BMC Plant Biol* 2008. **8**: 121.
30. **Thomas, T., Corcoran, L. M., Gugasyan, R., Dixon, M. P., Brodnicki, T.,** *et al*. Monocytic leukemia zinc finger protein is essential for the development of long-term reconstituting hematopoietic stem cells. *Genes Dev* 2006. **20**: 1175–1186.
31. **Thomas, T., Loveland, K. L. and Voss, A. K.,** The genes coding for the MYST family histone acetyltransferases, Tip60 and Mof, are expressed at high levels during sperm development. *Gene Expr Patterns* 2007. **7**: 657–665.
32. **McAllister, D., Merlo, X. and Lough, J.,** Characterization and expression of the mouse tat interactive protein 60 kDa (TIP60) gene. *Gene* 2002. **289**: 169–176.
33. **Sharma, M., Zarnegar, M., Li, X., Lim, B. and Sun, Z.,** Androgen receptor interacts with a novel MYST protein, HBO1. *J Biol Chem* 2000. **275**: 35200–35208.
34. **Akhtar, A. and Becker, P. B.,** Activation of transcription through histone H4 acetylation by MOF, an acetyltransferase essential for dosage compensation in *Drosophila*. *Mol Cell* 2000. **5**: 367–375.
35. **Bone, J. R., Lavender, J., Richman, R., Palmer, M. J., Turner, B. M. and Kuroda, M. I.,** Acetylated histone H4 on the male X chromosome is associated with dosage compensation in *Drosophila*. *Genes Dev* 1994. **8**: 96–104.
36. **Jeppesen, P. and Turner, B. M.,** The inactive X chromosome in female mammals is distinguished by a lack of histone H4 acetylation, a cytogenetic marker for gene expression. *Cell* 1993. **74**: 281–289.
37. **Gupta, A., Guerin-Peyrou, T. G., Sharma, G. G., Park, C., Agarwal, M.,** *et al*. The mammalian ortholog of *Drosophila* MOF that acetylates histone H4 lysine 16 is essential for embryogenesis and oncogenesis. *Mol Cell Biol* 2008. **28**: 397–409.
38. **Thomas, T., Dixon, M. P., Kueh, A. J. and Voss, A. K.,** Mof (MYST1 or KAT8) is essential for progression of embryonic development past the blastocyst stage and required for normal chromatin architecture. *Mol Cell Biol* 2008. **28**: 5093–5105.
39. **Taipale, M., Rea, S., Richter, K., Vilar, A., Lichter, P.,** *et al*. hMOF histone acetyltransferase is required for histone H4 lysine 16 acetylation in mammalian cells. *Mol Cell Biol* 2005. **25**: 6798–6810.
40. **Nguyen, D. K. and Disteche, C. M.,** Dosage compensation of the active X chromosome in mammals. *Nat Genet* 2006. **38**: 47–53.
41. **Smith, E. R., Eisen, A., Gu, W., Sattah, M., Pannuti, A.,** *et al*. ESA1 is a histone acetyltransferase that is essential for growth in yeast. *Proc Natl Acad Sci USA* 1998. **95**: 3561–3565.
42. **Clarke, A. S., Lowell, J. E., Jacobson, S. J. and Pillus, L.,** Esa1p is an essential histone acetyltransferase required for cell cycle progression. *Mol Cell Biol* 1999. **19**: 2515–2526.

43. **Kimura, A., Umehara, T. and Horikoshi, M.,** Chromosomal gradient of histone acetylation established by Sas2p and Sir2p functions as a shield against gene silencing. *Nat Genet* 2002. **32**: 370–377.
44. **Suka, N., Luo, K. and Grunstein, M.,** Sir2p and Sas2p opposingly regulate acetylation of yeast histone H4 lysine16 and spreading of heterochromatin. *Nat Genet* 2002. **32**: 378–383.
45. **Meijsing, S. H. and Ehrenhofer-Murray, A. E.,** The silencing complex SAS-I links histone acetylation to the assembly of repressed chromatin by CAF-I and Asf1 in *Saccharomyces cerevisiae. Genes Dev* 2001. **15**: 3169–3182.
46. **Bird, A. W., Yu, D. Y., Pray-Grant, M. G., Qiu, Q., Harmon, K. E.,** *et al.* Acetylation of histone H4 by Esa1 is required for DNA double-strand break repair. *Nature* 2002. **419**: 411–415.
47. **Kusch, T., Florens, L., Macdonald, W. H., Swanson, S. K., Glaser, R. L.,** *et al.* Acetylation by Tip60 is required for selective histone variant exchange at DNA lesions. *Science* 2004. **306**: 2084–2087.
48. **Ikura, T., Ogryzko, V. V., Grigoriev, M., Groisman, R., Wang, J.,** *et al.* Involvement of the TIP60 histone acetylase complex in DNA repair and apoptosis. *Cell* 2000. **102**: 463–473.
49. **Gorrini, C., Squatrito, M., Luise, C., Syed, N., Perna, D.,** *et al.* Tip60 is a haplo-insufficient tumour suppressor required for an oncogene-induced DNA damage response. *Nature* 2007. **448**: 1063–1067.
50. **Zhu, X., Singh, N., Donnelly, C., Boimel, P. and Elefant, F.,** The cloning and characterization of the histone acetyltransferase human homolog Dmel\TIP60 in *Drosophila melanogaster*: Dmel\TIP60 is essential for multicellular development. *Genetics* 2007. **175**: 1229–1240.
51. **Ceol, C. J. and Horvitz, H. R.,** A new class of *C. elegans* synMuv genes implicates a Tip60/NuA4-like HAT complex as a negative regulator of Ras signaling. *Dev Cell* 2004. **6**: 563–576.
52. **Frank, S. R., Parisi, T., Taubert, S., Fernandez, P., Fuchs, M.,** *et al.* MYC recruits the TIP60 histone acetyltransferase complex to chromatin. *EMBO Rep* 2003. **4**: 575–580.
53. **Fazzio, T. G., Huff, J. T. and Panning, B.,** An RNAi screen of chromatin proteins identifies Tip60-p400 as a regulator of embryonic stem cell identity. *Cell* 2008. **134**: 162–174.
54. **Miotto, B., Sagnier, T., Berenger, H., Bohmann, D., Pradel, J. and Graba, Y.,** Chameau HAT and DRpd3 HDAC function as antagonistic cofactors of JNK/AP-1-dependent transcription during *Drosophila* metamorphosis. *Genes Dev* 2006. **20**: 101–112.
55. **Burke, T. W., Cook, J. G., Asano, M. and Nevins, J. R.,** Replication factors MCM2 and ORC1 interact with the histone acetyltransferase HBO1. *J Biol Chem* 2001. **276**: 15397–15408.
56. **Miotto, B. and Struhl, K.,** HBO1 histone acetylase is a coactivator of the replication licensing factor Cdt1. *Genes Dev* 2008. **22**: 2633–2638.
57. **Aggarwal, B. D. and Calvi, B. R.,** Chromatin regulates origin activity in *Drosophila* follicle cells. *Nature* 2004. **430**: 372–376.
58. **Pak, D. T., Pflumm, M., Chesnokov, I., Huang, D. W., Kellum, R.,** *et al.* Association of the origin recognition complex with heterochromatin and HP1 in higher eukaryotes. *Cell* 1997. **91**: 311–323.
59. **Foss, M., McNally, F. J., Laurenson, P. and Rine, J.,** Origin recognition complex (ORC) in transcriptional silencing and DNA replication in *S. cerevisiae*. *Science* 1993. **262**: 1838–1844.
60. **Bell, S. P., Kobayashi, R. and Stillman, B.,** Yeast origin recognition complex functions in transcription silencing and DNA replication. *Science* 1993. **262**: 1844–1849.
61. **Micklem, G., Rowley, A., Harwood, J., Nasmyth, K. and Diffley, J. F.,** Yeast origin recognition complex is involved in DNA replication and transcriptional silencing. *Nature* 1993. **366**: 87–89.
62. **Voss, A. K., Thomas, T. and Gruss, P.,** Efficiency assessment of the gene trap approach. *Dev Dyn* 1998. **212**: 171–180.
63. **Rietze, R. L., Valcanis, H., Brooker, G. F., Thomas, T., Voss, A. K. and Bartlett, P. F.,** Purification of a pluripotent neural stem cell from the adult mouse brain. *Nature* 2001. **412**: 736–739.
64. **Merson, T. D., Dixon, M. P., Collin, C., Rietze, R. L., Bartlett, P. F.,** *et al.* The transcriptional coactivator Querkopf controls adult neurogenesis. *J Neurosci* 2006. **26**: 11359–11370.
65. **Scott, E. K., Lee, T. and Luo, L.,** Enok encodes a *Drosophila* putative histone acetyltransferase required for mushroom body neuroblast proliferation. *Curr Biol* 2001. **11**: 99–104.
66. **Katsumoto, T., Aikawa, Y., Iwama, A., Ueda, S., Ichikawa, H.,** *et al.* MOZ is essential for maintenance of hematopoietic stem cells. *Genes Dev* 2006. **20**: 1321–1330.
67. **Perez-Campo, F. M., Borrow, J., Kouskoff, V. and Lacaud, G.,** The HAT activity of MOZ is critical for the proliferation of hematopoietic precursors. *Blood* 2009. **113**: 4866–4874.
68. **Miller, C. T., Maves, L. and Kimmel, C. B.,** Moz regulates Hox expression and pharyngeal segmental identity in zebrafish. *Development* 2004. **131**: 2443–2461.
69. **Crump, J. G., Swartz, M. E., Eberhart, J. K. and Kimmel, C. B.,** Moz-dependent Hox expression controls segment-specific fate maps of skeletal precursors in the face. *Development* 2006. **133**: 2661–2669.
70. **Laue, K., Daujat, S., Crump, J. G., Plaster, N., Roehl, H. H.,** *et al.* The multidomain protein Brpf1 binds histones and is required for Hox gene expression and segmental identity. *Development* 2008. **135**: 1935–1946.
71. **Zhuravleva, J., Paggetti, J., Martin, L., Hammann, A., Solary, E.,** *et al.* MOZ/TIF2-induced acute myeloid leukaemia in transgenic fish. *Br J Haematol* 2008. **143**: 378–382.
72. **Pelletier, N., Champagne, N., Stifani, S. and Yang, X. J.,** MOZ and MORF histone acetyltransferases interact with the Runt-domain transcription factor Runx2. *Oncogene* 2002. **21**: 2729–2740.
73. **Akhtar, A., Zink, D. and Becker, P. B.,** Chromodomains are protein-RNA interaction modules. *Nature* 2000. **407**: 405–409.
74. **Gomez, E. B., Espinosa, J. M. and Forsburg, S. L.,** *Schizosaccharomyces pombe* mst2+ encodes a MYST family histone acetyltransferase that negatively regulates telomere silencing. *Mol Cell Biol* 2005. **25**: 8887–8903.
75. **Gomez, E. B., Nugent, R. L., Laria, S. and Forsburg, S. L.,** *Schizosaccharomyces pombe* histone acetyltransferase Mst1 (KAT5) is an essential protein required for damage response and chromosome segregation. *Genetics* 2008. **179**: 757–771.
76. **Kawahara, T., Siegel, T. N., Ingram, A. K., Alsford, S., Cross, G. A. and Horn, D.,** Two essential MYST-family proteins display distinct roles in histone H4K10 acetylation and telomeric silencing in trypanosomes. *Mol Microbiol* 2008. **69**: 1054–1068.
77. **McGraw, S., Morin, G., Vigneault, C., Leclerc, P. and Sirard, M. A.,** Investigation of MYST4 histone acetyltransferase and its involvement in mammalian gametogenesis. *BMC Dev Biol* 2007. **7**: 123.
78. **Smith, A. T., Tucker-Samaras, S. D., Fairlamb, A. H. and Sullivan, W. J. Jr.,** MYST family histone acetyltransferases in the protozoan parasite *Toxoplasma gondii. Eukaryot Cell* 2005. **4**: 2057–2065.
79. **Thompson, J. D., Higgins, D. G. and Gibson, T. J.,** CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. *Nucleic Acids Res* 1994. **22**: 4673–4680.
80. **Howe, L., Auston, D., Grant, P., John, S., Cook, R. G.,** *et al.* Histone H3 specific acetyltransferases are essential for cell cycle progression. *Genes Dev* 2001. **15**: 3144–3154.
81. **Borrow, J., Stanton, V. J., Andresen, J., Becher, R., Behm, F.,** *et al.* The translocation t(8;16)(p11;p13) of acute myeloid leukaemia fuses a putative acetyltransferase to the CREB-binding protein. *Nat Genet* 1996. **14**: 33–41.
82. **Haferlach, T., Kohlmann, A., Klein, H. U., Ruckert, C., Dugas, M.,** *et al.* AML with translocation t(8;16)(p11;p13) demonstrates unique cytomorphological, cytogenetic, molecular and prognostic features. *Leukemia* 2009. **23**: 934–943.
83. **Carapeti, M., Aguiar, R., Goldman, J. and Cross, N.,** A novel fusion between MOZ and the nuclear receptor coactivator TIF2 in acute myeloid leukemia. *Blood* 1998. **91**: 3127–3133.
84. **Chaffanet, M., Gressin, L., Preudhomme, C., Soenen-Cornu, V., Birnbaum, D. and Pebusque, M. J.,** MOZ is fused to p300 in an acute monocytic leukemia with t(8;22). *Genes Chromosomes Cancer* 2000. **28**: 138–144.
85. **Esteyries, S., Perot, C., Adelaide, J., Imbert, M., Lagarde, A.,** *et al.* NCOA3, a new fusion partner for MOZ/MYST3 in M5 acute myeloid leukemia. *Leukemia* 2008. **22**: 663–665.
86. **Liang, J., Prouty, L., Williams, B. J., Dayton, M. A. and Blanchard, K. L.,** Acute mixed lineage leukemia with an inv(8)(p11q13) resulting in fusion of the genes for MOZ and TIF2. *Blood* 1998. **92**: 2118–2122.

87. **Imamura, T., Kakazu, N., Hibi, S., Morimoto, A., Fukushima, Y.,** *et al.* Rearrangement of the MOZ gene in pediatric therapy-related myelodysplastic syndrome with a novel chromosomal translocation t(2;8)(p23;p11). *Genes Chromosomes Cancer* 2003. **36**: 413–419.
88. **Huntly, B. J., Shigematsu, H., Deguchi, K., Lee, B. H., Mizuno, S.,** *et al.* MOZ-TIF2, but not BCR-ABL, confers properties of leukemic stem cells to committed murine hematopoietic progenitors. *Cancer Cell* 2004. **6**: 587–596.
89. **Deguchi, K., Ayton, P. M., Carapeti, M., Kutok, J. L., Snyder, C. S.,** *et al.* MOZ-TIF2-induced acute myeloid leukemia requires the MOZ nucleosome binding motif and TIF2-mediated recruitment of CBP. *Cancer Cell* 2003. **3**: 259–271.
90. **Moore, S. D., Herrick, S. R., Ince, T. A., Kleinman, M. S., Dal Cin, P.,** *et al.* Uterine leiomyomata with t(10;17) disrupt the histone acetyltransferase MORF. *Cancer Res* 2004. **64**: 5570–5577.
91. **Panagopoulos, I., Fioretos, T., Isaksson, M., Samuelsson, U., Billstrom, R.,** *et al.* Fusion of the MORF and CBP genes in acute myeloid leukemia with the t(10;16)(q22;p13). *Hum Mol Genet* 2001. **10**: 395–404.
92. **Murati, A., Adelaide, J., Mozziconacci, M. J., Popovici, C., Carbuccia, N.,** *et al.* Variant MYST4-CBP gene fusion in a t(10;16) acute myeloid leukaemia. *Br J Haematol* 2004. **125**: 601–604.
93. **Vizmanos, J. L., Larrayoz, M. J., Lahortiga, I., Floristan, F., Alvarez, C.,** *et al.* t(10;16)(q22;p13) and MORF-CREBBP fusion is a recurrent event in acute myeloid leukemia. *Genes Chromosomes Cancer* 2003. **36**: 402–405.
94. **Kamine, J., Elangovan, B., Subramanian, T., Coleman, D. and Chinnadurai, G.,** Identification of a cellular protein that specifically interacts with the essential cysteine region of the HIV-1 Tat transactivator. *Virology* 1996. **216**: 357–366.
95. **Col, E., Caron, C., Chable-Bessia, C., Legube, G., Gazzeri, S.,** *et al.* HIV-1 Tat targets Tip60 to impair the apoptotic cell response to genotoxic stresses. *EMBO J* 2005. **24**: 2634–2645.