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Maf1 phenotypes and cell physiology[☆]

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Abstract

As a master regulator of transcription by RNA polymerase (Pol) III, Maf1 represses the synthesis of highly abundant non-coding RNAs as anabolic signals dissipate, as the quality or quantity of nutrients decreases, and under a wide range of cellular and environmental stress conditions. Thus, Maf1 responds to changes in cell physiology to conserve metabolic energy and to help maintain appropriate levels of tRNAs and other essential non-coding RNAs. Studies in different model organisms and cell-based systems show that perturbations of Maf1 can also impact cell physiology and metabolism. These effects are mediated by changes in Pol III transcription and/or by effects of Maf1 on the expression of select Pol II-transcribed genes. Maf1 phenotypes can vary between different systems and are sometimes conflicting as in comparisons between *Maf1* KO mice and cultured mammalian cells. These studies are reviewed in an effort to better appreciate the relationship between Maf1 function and cell physiology. This article is part of a Special Issue entitled: SI: Regulation of tRNA synthesis and modification in physiological conditions and disease edited by Dr. Boguta Magdalena.

Keywords

Maf1; RNA polymerase III; metabolism; cancer; futile cycle; tumor suppressor

1. Introduction

RNA polymerase (Pol) III has a unique and specialized role in eukaryotic cells in the transcription of short (~100–300 nucleotide long), highly stable and abundant non-coding RNAs. The majority of its products comprise tRNAs, the central adapter molecules for decoding mRNA sequences into proteins and the 5S rRNA component of ribosomes. Together these molecules account for ~20% of total cellular RNA and in rapidly growing cell populations their synthesis consumes ~15% of the nucleotides used in nuclear gene transcription [1]. Other Pol III transcripts have functions in pre-ribosomal RNA processing (RNase MRP RNA), 5′-end maturation of precursor tRNAs (RNase P RNA), protein secretion (7SL RNA) and pre-mRNA splicing (U6 snRNA), among others [2]. Indeed, the functional diversity of Pol III transcripts continues to grow with better understanding of their

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canonical activities (e.g. for Y RNAs and Vault RNAs, [3,4]) and with new research showing that many Pol III transcripts can be cleaved into smaller functional RNA molecules [5].

Transcription of a prototypical tRNA gene requires a six-subunit assembly factor (TFIIIC) for binding of intragenic promoter elements and for the subsequent stable assembly of a three-subunit initiation factor (TFIIIB) comprising the TATA box-binding protein TBP, the TFIIIB-paralog Brf1 and Bdp1 [6,7]. Recruitment of the 17 subunit polymerase is then followed by spontaneous promoter opening, nucleotide binding and initiation of transcription. Pol III genes with different promoter structures, such as 5S RNA genes and metazoan U6 snRNA genes, employ additional (TFIIIA) or different (SNAPc) factors for DNA binding and TFIIIB assembly, respectively [6,7]. A unique property of this system that is thought to be important for its high transcriptional output in vivo is the ability of a single polymerase molecule to initiate multiple rounds of transcription on the same gene. Biochemical studies indicate that this is achieved without dissociation of the polymerase from the DNA in a process termed facilitated recycling [8,9]. Whether facilitated recycling occurs in vivo is not known. Nevertheless, efficient repression of these short, actively transcribed genes can best be achieved by targeting the initiation step of transcription. In doing so, the conservation of metabolic energy is maximized while ensuring that macromolecular constituents stay in balance. Research on the regulation of Pol III transcription from yeast to mammals shows that targeting of the initiation step is a conserved strategy. Biochemical and genetic studies have led to a model of regulation in which posttranslational changes to TFIIIB, Pol III and Maf1, the master negative regulator in this system, effect rapid repression of Pol III transcription in response to diverse nutritional, environmental and cellular stress conditions (reviewed in [10] and other articles in this issue). A key feature of this model is the absolute requirement for Maf1 [11] and its direct interaction with the transcription machinery, in particular, Pol III [12].

For reasons of experimental convenience, studies on the regulation of Pol III transcription often compare cells growing optimally to those that have been exposed to an acute repressing condition [1,11,13,14]. This produces rapid and quantitatively large decreases in RNA synthesis. While there may be little doubt about the experimental outcome of such treatments, the conditions are generally non-physiologic and the results can give the impression that regulation is achieved by a switch-like (on or off) mechanism. This is unlikely to be the case since Pol III output must be able to satisfy biosynthetic requirements under widely variable growth rates. These growth rates reflect the quality and quantity of nutrients, growth factors, toxicants, genetic perturbations and cell type and produce changes in cell physiology through their action on conserved nutrient and stress signaling pathways (see [1,10,13] and this issue).

Just as changes in cell physiology lead to adjustments in Pol III transcription in order to balance the production of RNA with cellular demand, research over the last several years has found that perturbations of the Maf1 repressor can reciprocally impact cell physiology and metabolism. This review examines these findings and discusses the similarities and differences among the various model organisms and cell-based systems in which this work has been conducted.

2. Metabolic connections to Pol III and Maf1 in yeast

2.1. Mitochondrial function

One of the phenotypes associated with gene-deletion and severe loss of function mutations in *MAF1* in yeast is diminished growth on non-fermentable carbon sources at 30°C (e.g. glycerol, lactate and ethanol) and lethality on these media when the temperature is raised to 37°C [12,15–17]. This phenotype points to a defect in mitochondrial function but the relationship to Pol III transcription and Maf1 has been enigmatic. Studies suggest that the increased amount of total tRNA in *maf1* cells on glycerol is phenotypically important since the lethality at 37°C can be suppressed by deleterious mutations in Pol III subunits that reduce the size of the total tRNA population [12,17]. One explanation for the implied functional deficit of *maf1* mitochondria has centered on two nuclear-encoded tRNA species, tRNA^{Lys}CUU and tRNA^{Gln}, that are imported into yeast mitochondria and function in mitochondrial translation [18,19]. Compromised function of either of these tRNAs could impact mitochondrial protein synthesis but it is not clear how their expression in *maf1* cells could result in a functional deficit. A possible solution to this conundrum may lie in the recent finding that loss of Maf1 can lead to hypomodification of some tRNAs [20]. Specifically, the N2,N2 dimethyl G26 modification catalyzed by the Trm1 methyl transferase was shown to be substantially reduced in many different tRNAs in an *S. pombe maf1* strain [20]. Importantly, the undermodification of one of these tRNAs (tRNA^{Ser}UCA) was found to be functionally significant, reducing its translational efficiency in a nonsense suppression assay. Thus, tRNA hypomodification explains the paradoxical loss of nonsense suppressor activity associated with defective repression of Pol III transcription in *maf1* mutant strains [21]. Interestingly, the tRNA^{Lys}CUU species imported into mitochondria in *S. cerevisiae* was among a group of *S. pombe* tRNAs that were highly undermodified for N2,N2 dimethyl G26 [20] suggesting that the function of this tRNA could be negatively affected. More direct experiments are needed to assess mitochondrial function and tRNA^{Lys}CUU activity in *maf1* yeast.

2.2. Gluconeogenesis

Recent studies in *S. cerevisiae* suggest that the conditional growth phenotype of *maf1* strains on non-fermentable carbon sources may be more complex than suggested by the preceding observations. Transcriptional profiling of wild-type and *maf1* yeast on glycerol following a shift to 37°C identified a handful of protein coding genes whose expression was lower in the mutant strain [22]. Two of the affected genes, *FBP1* and *PCK1*, encode key gluconeogenic enzymes (fructose 1,6 bisphosphatase and phosphoenolpyruvate carboxykinase, respectively) whose expression is repressed by glucose and induced on non-fermentable carbon sources. However, in the *maf1* strain, the induction of both genes on glycerol is dramatically reduced. The main pathway for glycerol catabolism in *S. cerevisiae* proceeds in three steps with a glycerol/H⁺ symporter, glycerol kinase and glycerol-3-phosphate dehydrogenase yielding dihydroxyacetone phosphate [23] which enters glycolysis below fructose 1,6 bisphosphate (Fig. 1A). Since strains deleted for *FBP1* are unable to grow on glycerol [24] and *maf1* cells are defective in inducing *FBP1* on glycerol, it appears that efficient growth on this medium requires the flux of carbon atoms into hexose and/or pentose sugars in the upper part of glycolysis. Although the metabolic importance of this

flux needs to be determined, the upstream metabolites are known to participate in inositol, mannose, trehalose and glycogen synthesis and provide substrates for the pentose phosphate pathway (Fig. 1A). As noted above, the temperature-sensitive lethality of the *maf1* strain on glycerol can be rescued by hypomorphic mutations in Pol III. Thus, it appears that simply slowing down Pol III transcription or a process that is affected by this transcription is critical for restoring viability.

Mechanistic studies have so far not identified how Maf1 functions in the derepression/induction of *FBP1* and *PCK1* on glycerol. One possibility is that the expression of a shared transcriptional activator (e.g. Cat, Sip4) needed for induction of these genes on glycerol might be compromised at the translational level due to tRNA hypomodification [20]. Alternatively, it is possible that Maf1 may function in this unique context as a co-activator for Pol II in the transcription of *FBP1* and *PCK1*. The ability of Maf1 to affect the transcription of a small number of protein coding genes has been known for some time based on knockdown and overexpression studies in mammalian cells and in *C. elegans* [25–27](see below). However, in all of these cases, Maf1 functions as a repressor, in line with its role in the Pol III system. More recently, evidence has emerged that mammalian Maf1 may function as co-activator of transcription of the *PTEN* tumor suppressor [28](see also in this issue).

2.3. A metabolic enzyme associated with Pol III and its genes

Another link between carbon metabolism and Pol III involves *FBA1*, a gene dosage suppressor of a loss of function mutation (*rpc128-1007*) in the second largest subunit of Pol III [29]. *FBA1* encodes fructose 1,6-bisphosphate aldolase, an enzyme common to glycolysis and gluconeogenesis that catalyzes the reversible conversion of fructose 1,6 bisphosphate to glyceraldehyde-3-P and dihydroxyacetone-P (Fig. 1A). The *rpc128-1007* allele is cold-sensitive due to a Gly1007Ala mutation at a buried interface between Rpc128 and AC40, a subunit that is shared between Pools I and III (Fig. 1B) [30]. The position of this mutation in the structure of Pol III suggests a specific assembly/stability defect. Consistent with this, *FBA1* overexpression did not suppress conditional phenotypes resulting from mutations in other Pol III subunits (Rpc160, Rpc31 and Rpc11) and Pol III subunit abundance was significantly reduced in whole cell extracts and in Pol III immunoprecipitates from the Rpc128 mutant strain [31]. *FBA1* suppression of the *rpc128-1007* cold-sensitive growth phenotype increased the low level of precursor and mature tRNAs in these cells. Notably, increased growth of the suppressed strain was not dependent on Fba1 catalytic activity consistent with a structural role for Fba1 in stabilizing the mutant polymerase. In keeping with this view, a fraction of Fba1 is localized to the nucleus and the protein interacts with Pol III in crude extracts. Independent studies have also found Fba1 as a minor component in numerous Pol III subunit TAP-tag purifications although Fba1 is not a frequent contaminant of purifications using this method [32]. The finding that a human aldolase isoform is also nuclear and ChIPs to a number of Pol III-transcribed genes further strengthens the functional importance of this association [29].

What function could Fba1 be providing though its interaction with Pol III? Unlike the *rpc128-1007* mutant polymerase, the wild-type enzyme is unlikely to require stabilization at

its interface with AC40. Similarly, unlike the mutant polymerase which does not require Fba1 catalytic activity for suppression, the normal function of Fba1 in Pol III gene transcription may depend on this activity or its ability to bind substrates. Notably, one of its substrates, fructose 1,6 biphosphate (F-1,6-P), is an allosteric activator of pyruvate kinase, the terminal enzyme in glycolysis and a critical regulator of glycolytic flux [33,34](Fig. 1A). Accordingly, it is possible that the binding of this key metabolite to Fba1 may function as a metabolic sensor to tune, in some unknown manner, the activity of Pol III with metabolic conditions (Fig. 1C). Support for the feasibility of this idea emerged recently with the demonstration that F-1,6-P and aldolase mediate glucose sensing by AMPK in mammals [35].

3. Impact of Maf1 on cell physiology in metazoans

3.1. *Drosophila* Maf1 controls organismal growth through systemic effects of altered translation

As the first multicellular organism in which Maf1 function was examined, studies with transgenic *Drosophila* provided numerous insights as well as unexpected results [36]. Ubiquitous expression of a Maf1-targeted RNAi during development increased tRNA synthesis including the levels of initiation methionine tRNA ($\text{tRNA}_i^{\text{Met}}$). Along with this, larval growth was stimulated, development from egg laying to pupation was accelerated and pupal volume (a measure of adult body size) was increased (Table 1). On the assumption that $\text{tRNA}_i^{\text{Met}}$ might be limiting for growth in this system, independent transgenic lines were generated containing one extra copy of the $\text{tRNA}_i^{\text{Met}}$ gene. Fly larvae overexpressing $\text{tRNA}_i^{\text{Met}}$ contained more polysomes than control larvae and phenocopied the accelerated growth and increased body size seen upon Maf1 knockdown, consistent with increased protein synthesis and animal growth. However, rather than representing primarily a cell autonomous effect, subsequent experiments showed that Maf1 knockdown specifically in the larval fat body could accelerate development and increase body size [36]. Conversely, fat body-specific inhibition of Pol III had the opposite effect [37,38]. In *Drosophila*, the larval fat body is the functional equivalent of vertebrate liver and white adipose tissue and serves as an endocrine organ coupling nutrient levels to the release of insulin-like peptides in the brain [39]. This inter-organ communication is mediated by nutrient-dependent fat body secretion of numerous protein/peptide ligands [39]. Accordingly, observed changes in the expression of insulin-like peptides and insulin receptor in peripheral tissues after specific knockdown of *Maf1* in the fat body were consistent with increased tRNA synthesis and ligand secretion from this tissue. Thus, the major phenotypes of *Maf1* knockdown in *Drosophila* result from non-cell-autonomous changes in insulin signaling.

3.2. *C. elegans* Maf1 – diverse readouts from reproductive fitness to lifespan extension

Studies on Maf1 in *C. elegans* have employed overexpression and knockdown approaches to assess phenotypes and molecular changes in the whole animal. Both perturbations produce approximately two fold changes in Maf1 expression at the RNA level [27]. As in other systems, Maf1 is limiting in *C. elegans*; overexpression reduced the levels of all measured precursor and mature tRNAs. The opposite response was generally seen upon *Maf1* knockdown although not all of the tested tRNAs were affected. (This may reflect the relative

resistance of some tRNA genes to Maf1-mediated repression as found recently in mammalian cells and yeast [40,41]). Overexpression of Maf1 also reduced the expression of a surprisingly large number of Pol II-transcribed genes. Gene Ontology (GO) terms enriched in a set of 1495 downregulated genes included reproduction, metabolism and lipid transport processes and led to targeted experiments in these areas. Interestingly, the downregulated lipid transport genes included several vitellogenin (*vit*) yolk proteins which, in the worm, are secreted from the intestine, diffuse to the gonad and are endocytosed by developing oocytes. Decreased *vit* gene expression was independently confirmed and a transgenic *VIT-2::GFP* fusion revealed the expected changes in protein abundance in response to Maf1 levels. These observations suggested that Maf1 overexpression might result in a reproductive phenotype and indeed, a significant reduction in fecundity (brood size) was noted (Table 1). Thus, the experiments demonstrate a non-cell-autonomous phenotype due to enhanced Maf1 expression. Additionally, since the effect of Maf1 overexpression on *vit* gene expression could not be phenocopied by direct knockdown of Pol III transcription (e.g. using RNAi against Brf1), the data are consistent with findings in mammalian cells that Maf1 can function as a repressor of a select group of Pol II-transcribed genes [25,26]. Indeed, specific functional overlap has been noted between *C. elegans* and mammalian Maf1 concerning the expression of the TATA-box binding factor TBP and the transcription of lipid metabolic genes that impact fat accumulation (see below).

Phenotypes related to lifespan in *C. elegans* have been examined using a *Maf1* transposon insertion mutant, *Mafr-1(tm6082)* [42]. This line of inquiry was prompted by the knowledge that treatments known to extend lifespan in various organisms (e.g. TOR inhibition and calorie restriction) lead to Maf1-dependent repression [11,14,43] while deletion of *Maf1* in yeast reduces chronological lifespan [42,44]. The *tm6082* allele removes the phylogenetically conserved box C region, including three of the four β -sheets that make up the central core of Maf1 along with additional sequences (a total of 79 amino acids) and introduces 37 different amino acids in their place [42,45,46]. Interestingly, rather than shortening lifespan, the *tm6082* mutant extended lifespan under both normal and calorie-restricted conditions. This finding was confirmed by RNAi knockdown of *Maf1*. Lifespan extension in the *tm6082* mutant was also suppressed by Maf1 overexpression demonstrating that the phenotype is due to a loss of Maf1 function. Curiously, other phenotypes identified using the *tm6082* allele in a different study were interpreted as resulting from a gain of function [45]. It is not clear how to reconcile these observations as neither study examined the levels of Maf1 mRNA or protein in the *tm6082* mutant. Several traits associated with the extension of lifespan in model organisms were also affected in *tm6082* mutant worms. These included an enhanced stress response, induction of the mitochondrial unfolded protein response and upregulation of autophagy (Table 1) [42]. Notably, elevated autophagy and lifespan extension were also reported for a whole body *Maf1* knockout in mice [47].

3.3. Mammalian Maf1 is an unconventional tumor suppressor

Initial studies on human Maf1 in cell lines demonstrated that its effect on Pol III transcription and its regulation by nutrients, alkylating DNA damage and mTOR signaling was fundamentally conserved based on earlier findings in yeast [14,48]. However, in studies with glioblastoma cells, overexpression and knockdown of Maf1 was unexpectedly found to

also affect rDNA transcription by Pol I [25]. These changes were attributed to an indirect effect of Maf1 on the expression of TBP which is an essential component of the Pol I selectivity factor SL1. Subsequent resection of the TBP promoter mapped a Maf1 responsive region to an Elk-1 binding site proximal to the transcription start site. Although Maf1 is not a DNA binding protein, its occupancy of the TBP promoter varied with Maf1 expression and was reciprocal with Elk-1. Similar results were obtained for another Elk-1 regulated gene (*Egr-1*) but not other protein coding genes that were tested at the time. These data provided the first evidence that Maf1 functions as a repressor of a subset of genes transcribed by Pol II (Table 1) [25]. Additional examples have come to light more recently after it was learned that Maf1 expression is anti-correlated with key lipogenic genes (*Fasn* and *Acc1*) in the liver of mice fed a high carbohydrate diet [26]. Subsequently, Maf1 knockdown or overexpression in transformed hepatocytes or hepatocarcinoma cells showed reciprocal changes in the expression of *Fasn* and *Acc1* but not other lipid regulators such *Srebp1c*. As with the TBP promoter, a region of the *Fasn* promoter proximal to the transcription start site was responsive to changes in Maf1 levels in reporter assays and Maf1 was shown to associate with this region by chromatin immunoprecipitation. Thus, the results indicate that Maf1 functions as a direct transcriptional repressor of genes whose expression is limiting for lipogenesis. The functional significance of this repression was demonstrated by histological analysis of lipid accumulation in hepatocarcinoma cells following Maf1 knockdown and by the decrease in liver triglycerides in mice on a high carbohydrate diet upon ectopic overexpression of Maf1 (Table 1). As alluded to earlier, this novel Pol II-related function of Maf1 is conserved in *C. elegans* where Maf1-dependent changes in the expression of *Fasn* and *Acc1* also affect the production and storage of lipids [27].

Given that elevated levels of TBP have been shown to promote cell transformation [49] and that increased tRNA and rRNA synthesis are centrally involved in cell transformation and tumorigenesis [50–52], the ability of Maf1 overexpression to repress these processes suggested its potential as a tumor suppressor. Indeed, stable overexpression of Maf1 in PTEN-deficient glioblastoma cells reduced the ability of the cells to form colonies in soft agar (i.e. suppressed cell transformation) without affecting cell doubling time [25]. These findings have since been extended in studies that examined Maf1 expression and effects in normal and PTEN-deficient mouse liver and prostate tissue, human liver and prostate cancer, liver cancer cell lines and xenografts (Table 1) [26,28]. Low levels of Maf1 are found in liver and prostate cancer versus normal tissue and are associated with disease progression and poor prognosis in hepatocellular carcinoma. These outcomes likely reflect the fact that Maf1 expression is controlled by PI3K-AKT signaling [53]. Thus, the frequent upregulation of PI3K-AKT signaling in cancer due, for example, to loss of the *PTEN* tumor suppressor, leads to a reduction in Maf1 protein that is mediated by FoxO1 and potentially other mechanisms [26]. Conversely, overexpression of Maf1 in various liver cancer cell lines was found to inhibit colony formation and invasiveness and to dramatically reduce tumor growth in xenografts [26,28]. Interestingly, the striking ability of Maf1 to suppress tumorigenesis when overexpressed is not mirrored by a tumorigenic phenotype in mice that lack Maf1 from conception. *Maf1* KO mice are not tumor prone and as already mentioned, have an extended lifespan [47]. In line with these findings, Maf1 is infrequently mutated in human cancer and is not currently considered to be a cancer driver gene [54,55]. Nonetheless, given

the biological effects of Maf1 over-expression, it will be both interesting and important to learn about the processes impacted and the mechanisms employed by this unconventional tumor suppressor.

3.4. Maf1 KO mice — a model of metabolic inefficiency

Mice that are homozygous for a complete deletion of the Maf1 coding sequence are viable and fertile although breeding of homozygous animals reveals that fertility and fecundity are slightly reduced [47]. Reduced fecundity was also observed in *C. elegans* although in this case it was observed upon *Maf1* overexpression (Table 1) [27]. Thus, the same phenotype is obtained by opposing effects on Maf1. Presumably, the underlying mechanisms are likely to be entirely different in these organisms. *Maf1* KO mice are resistant to weight gain on normal and high fat diets and are significantly leaner than their wild-type counterparts. This resistance to weight gain is due to loss of the Maf1 protein as opposed to an indirect effect of the gene deletion since independent *Maf1* null lines containing either a single base insertion or an eight base deletion after amino acid 64 are also resistant to weight gain [47]. Three properties of the mice that likely contribute to their lower body weight and lean body composition have been identified: (i) food intake of *ad libitum*-fed, weight-matched animals is reduced; (ii) metabolic efficiency is decreased and (iii) *Maf1* KO mice are slightly smaller than wild-type mice based on body length measurements at 24 weeks of age [47]. Efforts to understand the molecular basis of these complex traits are still at an early stage. However, current findings indicate that metabolic inefficiency is an important factor in the resistance of *Maf1* KO mice to obesity and non-alcoholic fatty liver disease.

Maf1 KO mice are metabolically inefficient; they waste metabolic fuel and thus have fewer excess calories available for storage as fat. Evidence supporting this view at the whole body level was provided by the lower body weight and lower percentage of body fat of *Maf1* KO mice versus control mice during paired-feeding on a high fat diet (i.e. equal daily caloric intake) [47]. Thus, *Maf1* KO mice are less efficient at converting the food they consume into biomass. Indirect calorimetry studies showed that energy expenditure is elevated in *Maf1* KO mice throughout the diurnal cycle with the largest differences occurring during the night when mice are more active. Importantly, the activity of *Maf1* KO mice was not distinguishably different from wild-type mice suggesting that their enhanced energy expenditure has a metabolic basis. Experimental support for this view has been obtained in several tissues including liver, skeletal muscle and white adipose tissue (WAT).

In white adipose tissue from *Maf1* KO mice, the basal level of lipolysis is increased relative to wild-type along with the amount of activated (phosphorylated) hormone-sensitive lipase (HSL), a key lipolytic enzyme (Table 1) [47]. These data are consistent with an increase in energy demand in the whole animal that is addressed in WAT through the production of fatty acids for oxidative tissues (Fig. 2). Notably, expression profiling of WAT from fasted mice did not identify any significant changes in the Pol II transcriptome. Thus, post-transcriptional and/or post-translational mechanisms are likely involved in signaling the metabolic changes in this tissue. The observed activation of HSL which is mediated in WAT by protein kinase A in response to β 3-adrenergic receptor signaling supports this view [56].

Lipid metabolism is also affected in the liver where fatty acid synthesis is modestly increased. Despite this, liver triglyceride levels are dramatically lower (Table 1). The explanation for this apparent disparity is provided by an increase in autophagy and lipophagy as evidenced by (i) the increased flux of the autophagic marker LC3-II to lysosomes; (ii) the increased association of LC3-II with the lipid droplet fraction in liver homogenates and (iii) the increased association of LC3 with lipid droplets determined by immunofluorescence [47]. These changes provide further evidence of the increased demand for cellular energy in *MafI* KO mice (Fig. 2). In the liver, this demand appears to be met by the increased delivery of lipids and proteins to lysosomes for catabolism by lipases and proteases in order to produce substrates for the TCA cycle and oxidative phosphorylation.

Targeted metabolite profiling of fasted liver and skeletal muscle supports the increase in autophagy, revealing elevated levels of many amino acids and their downstream metabolites including the polyamine spermidine, a known inducer of autophagy [57]. More recently, the extension of targeted metabolomics to the fed state in liver has revealed even more dramatic changes than in the fasted state including quantitatively larger increases in amino acid levels, large reductions in the levels of many long chain acylcarnitine species and increases in short and medium chain acylcarnitines (I. Willis, unpublished data). Since the carnitine shuttle is responsible for transporting long chain fatty acids into mitochondria for β -oxidation, the lower levels of long chain acylcarnitines in fed *MafI* KO liver demonstrate an increase in energy demand that is not adequately met by the ingested food (Fig. 2). Thus, consistent with the relative greater energy expenditure differential between wild-type and *MafI* KO mice during active feeding, it appears that *MafI* KO mice derive a significant amount of this energy by breaking down and oxidizing fat.

3.5. What is driving the demand for energy in *MafI* KO mice?

One early consideration was that metabolic energy might be wasted through increased uncoupling of the mitochondrial proton gradient from ATP synthesis. However, the level of the uncoupling protein UCP1 was not affected, the mice had a normal body temperature and were not resistant to cold stress and there was no evidence of browning in white adipocytes [47,58]. Insight into the origin of the energy demand came from the seemingly unlikely possibility that Pol III transcription might be responsible. Initial experiments found that Pol III occupancy in fasted liver from *MafI* KO mice was increased on average ~four-fold for active Pol III-transcribed genes [59] and comparable increases were seen by northern blotting of liver RNA for several short-lived pre-tRNA species [47]. Indeed, by this measure, Pol III transcription was increased in nine different tissues to varying degrees, up to almost an order of magnitude in some cases. In contrast, the levels of individual mature tRNAs in the different tissues were essentially unchanged as was the amount of total tRNA as a fraction of total RNA. Thus, the increased synthesis of tRNA must have been largely matched by increased tRNA turnover and the concept of a futile cycle of tRNA synthesis and turnover emerged as a mechanism for energy expenditure (Fig. 2) [47].

In yeast cells growing in rich medium with a short generation time (~90 min), tRNA synthesis appears to operate at close to maximal capacity and deletion of *MafI* results in minimal, if any, increase in transcription [11]. It was surprising therefore that an

examination of precursor and mature tRNA levels and Pol III occupancy in mice that had been fasted and refed yielded similar findings as in the fasted state (Bonhoure et al. submitted). For most active loci, Pol III occupancy in fed liver was found to be substantially higher in *Maf1* KO versus wild-type tissue. Remarkably, these differences were quantitatively larger than in the fasted state. The occupancy of Pol III-transcribed genes in fed versus fasted *Maf1* KO liver was also much higher in the fed state. Thus, while Pol III occupancy decreases in the fed to fasted transition as the mice switch from using glucose as the primary fuel source to lipids, it is evident that Maf1 functions chronically to limit the association of Pol III with its genes. This chronic activity of Maf1 is not restricted to postmitotic cells in the liver. Increases in Pol III occupancy and corresponding increases in nascent RNA synthesis have been reported in human IMR90hTert cells growing in serum replete medium following Maf1 knockdown [40]. In addition, pre-tRNA levels are similarly elevated in fed and fasted conditions across multiple *Maf1* KO tissues with the exception of liver where the increase was lower (perhaps reflecting more active turnover of these transcripts) (Bonhoure et al. submitted). In summary, the data suggest that Pol III transcription in *Maf1* KO mice is likely increased in all nucleated cells throughout the diurnal cycle with the most substantial increases occurring upon feeding. Higher levels of Pol III transcription in fed versus fasted *Maf1* KO mice are thought to be achieved by pro-growth signaling to the transcription machinery that is now unopposed by the absence of Maf1 (see [10] and other articles in this volume). The findings lend support to the idea that constitutively high levels of Pol III transcription provide a mechanism for wasting significant amounts of metabolic energy and contribute to the lean phenotype of mice with a whole body knockout of *Maf1*.

3.6. Translational effects in *Maf1* KO mice

Initial analysis of polysome profiles in liver extracts from fed and fasted *Maf1* KO mice indicated that translation was diminished in the fed state relative to wild-type mice (Bonhoure et al. submitted). Consistent with this, puromycin incorporation into nascent liver proteins was also lower. Subsequently, a modest global reduction of protein synthesis in *Maf1* KO liver was confirmed by ribosome profiling with spike-in normalization against *Drosophila* S2 cell extracts. This experiment also provided insight into the underlying mechanism since ribosomal proteins and other translation factors that are characterized by mRNAs with 5' terminal oligopyrimidine (TOP) sequences were enriched among the mRNAs whose translation efficiency scores were significantly lower in the knockout (Bonhoure et al. submitted). Lower rates of translation in *Maf1* KO mice may, over time, contribute to their slightly smaller body size [47].

4. Phenotypic differences of *Maf1* depletion or loss in cells and model organisms

Despite the common function of Maf1 as a repressor of Pol III transcription in eukaryotes, the phenotypes observed upon knockout or knockdown of Maf1 can produce unique and disparate outcomes in different organisms and contexts (Table 1). For example, the ability of Maf1 to affect transcription by Pol I has been noted in glioblastoma cells but was not observed in *Maf1* KO liver. Similarly, the effects of *Maf1* knockdown on the expression of

certain protein coding genes (e.g. TBP and the lipogenesis genes *Fasn* and *Acc1*) in *C. elegans* and various mammalian cell lines were not apparent by transcriptome profiling of WAT or liver or ribosome profiling of liver from *Maf1* KO mice. Despite this, increased fatty acid synthesis was reported in all of these systems but the effects of knockdown versus knockout on fat accumulation were diametrically opposed. Maf1 has also been reported to function as a direct activator of *PTEN* gene transcription in liver cancer cell lines [28] but *PTEN* expression at the RNA and protein level was not affected in the liver of *Maf1* KO mice (Bonhoure et al. submitted). Additionally, differences in organismal growth and translation are apparent when comparing *Maf1* phenotypes in *Drosophila* and mice. Any of a number of different explanations may account for the contrasting phenotypes in these cases. Among the possibilities, there may be intrinsic differences in the functions or effects of Maf1 in divergent species. For example, increased tRNA_i^{Met} in flies, especially in the fat body, but not in mouse tissues is consistent with the increase in translation and growth in the former and not the latter. Phenotypic differences may also reflect partial versus complete loss of Maf1 function, comparisons involving differentiated versus proliferative or transformed cells and effects of Maf1 loss on developmental processes that may impact postnatal phenotypes. Metabolic effects of the culture/growth media versus animal physiology may also be an important variable. Our current data suggest that the metabolism of amino acids and lipids is significantly affected in *Maf1* KO mice as a consequence of a shift in energy balance due, at least in part, to futile RNA synthesis (Fig. 2). Given these metabolic changes in the mouse, it seems possible that perturbations of cellular metabolism due to cell transformation and/or growth conditions could mask some phenotypes or uncover others. In support of this idea, altered metabolism is a well-known hallmark of cancer [60] and cancer cells growing in culture are known to exhibit different nutrient dependencies (e.g. for glutamine and cysteine) and metabolic phenotypes (e.g. drug sensitivity) than their progenitors in vivo [61–63]. In any event, understanding the molecular basis of the phenotypes resulting from Maf1 perturbation in cell-based systems and in animal models is likely to contribute important and fundamental knowledge that may help inform therapeutic opportunities in cancer and metabolic disease.

5. Concluding remarks

As demonstrated most clearly in the comparative phenotypic analysis of *Maf1* KO mice and *Maf1* knockdown in mammalian cells, context is critical. However, the specific contextual parameters that contribute to phenotype are not well-defined. Nonetheless, future studies should attempt to address the disparities with different approaches. One way to do this involves using tissue-specific *Maf1* KO mice to identify cell autonomous and non-cell-autonomous effects. Although the lean phenotype of *Maf1* KO mice is thought to require contributions from multiple tissues [47], whole body metabolism is not just the sum of its parts; inter-organ metabolic pathways and sympathetic nervous system innervation of peripheral organs are also key contributors. Given that non cell-autonomous phenotypes of Maf1 have already been described in *Drosophila* and *C. elegans*, it seems likely that this will be the case, to some extent, in the mouse.

For cancer-related phenotypes, it will be important to move beyond cell lines and xenografts and manipulate Maf1 expression in genetically-defined animal models of cancer. These

models require a significant investment of time and resources to develop. However, the results should be definitive and will allow mechanistic studies in a physiologically-relevant context. Concerning Maf1 effects on Pol II transcription, a key question is how Maf1 is recruited to target promoters? Convention would suggest specific protein-protein interactions with promoter-selective transcription factors, although no candidate factors have been identified thus far. An alternate possibility is that Maf1 may affect Pol II transcription either locally [64,65] or through topological domains involving long range DNA loop interactions [66]. Recent work with human monocytes has identified more than 250 direct tDNA contact domains and tDNA clusters. These domains are dynamic and connect multiple tDNAs to one another and to protein-coding genes that exhibit correlated patterns of gene expression during macrophage differentiation. Thus, it may be informative to examine whether Maf1-regulated Pol II-transcribed genes are associated with tRNA genes in topological domains.

Transparency document

The Transparency document associated with this article can be found, in online version.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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References

1. Moir RD, Willis IM. Regulation of pol III transcription by nutrient and stress signaling pathways. *Biochim Biophys Acta*. 2013; 1829:361–375. [PubMed: 23165150]
2. Dieci G, Fiorino G, Castelnovo M, Teichmann M, Pagano A. The expanding RNA polymerase III transcriptome. *Trends Genet*. 2007; 23:614–622. [PubMed: 17977614]
3. Kowalski MP, Krude T. Functional roles of non-coding Y RNAs. *Int J Biochem Cell Biol*. 2015; 66:20–29. [PubMed: 26159929]
4. Li F, Chen Y, Zhang Z, Ouyang J, Wang Y, Yan R, Huang S, Gao GF, Guo G, Chen JL. Robust expression of vault RNAs induced by influenza A virus plays a critical role in suppression of PKR-mediated innate immunity. *Nucleic Acids Res*. 2015; 43:10321–10337. [PubMed: 26490959]
5. Kumar P, Kuscu C, Dutta A. Biogenesis and function of transfer RNA-related fragments (tRFs). *Trends Biochem Sci*. 2016; 41:679–689. [PubMed: 27263052]
6. Geiduschek EP, Kassavetis GA. The RNA polymerase III transcription apparatus. *J Mol Biol*. 2001; 310:1–26. [PubMed: 11419933]
7. Schramm L, Hernandez N. Recruitment of RNA polymerase III to its target promoters. *Genes Dev*. 2002; 16:2593–2620. [PubMed: 12381659]
8. Dieci G, Sentenac A. Facilitated recycling pathway for RNA polymerase III. *Cell*. 1996; 84:245–252. [PubMed: 8565070]
9. Arimbasseri AG, Rijal K, Maraia RJ. Comparative overview of RNA polymerase II and III transcription cycles, with focus on RNA polymerase III termination and reinitiation. *Transcription*. 2014; 5:e27369.
10. Willis IM, Moir RD. Signaling to and from the RNA polymerase III transcription and processing machinery. *Annu Rev Biochem*. 2018

11. Upadhy R, Lee J, Willis IM. Maf1 is an essential mediator of diverse signals that repress RNA polymerase III transcription. *Mol Cell*. 2002; 10:1489–1494. [PubMed: 12504022]
12. Pluta K, Lefebvre O, Martin NC, Smagowicz WJ, Stanford DR, Ellis SR, Hopper AK, Sentenac A, Boguta M. Maf1p, a negative effector of RNA polymerase III in *Saccharomyces cerevisiae*. *Mol Cell Biol*. 2001; 21:5031–5040. [PubMed: 11438659]
13. Boguta M. Maf1, a general negative regulator of RNA polymerase III in yeast. *Biochim Biophys Acta*. 2013; 1829:376–384. [PubMed: 23201230]
14. Michels AA, Robitaille AM, Buczynski-Ruchonnet D, Hodroj W, Reina JH, Hall MN, Hernandez N. mTORC1 directly phosphorylates and regulates human MAF1. *Mol Cell Biol*. 2010; 30:3749–3757. [PubMed: 20516213]
15. Murawski M, Szczepaniak B, Zoladek T, Hopper AK, Martin NC, Boguta M. maf1 mutation alters the subcellular localization of the Mod5 protein in yeast. *Acta Biochim Pol*. 1994; 41:441–448. [PubMed: 7732762]
16. Moir RD, Lee J, Haeusler RA, Desai N, Engelke DR, Willis IM. Protein kinase A regulates RNA polymerase III transcription through the nuclear localization of Maf1. *Proc Natl Acad Sci U S A*. 2006; 103:15044–15049. [PubMed: 17005718]
17. Cieła M, Towpik J, Graczyk D, Oficjalska-Pham D, Harismendy O, Suleau A, Balicki K, Conesa C, Lefebvre O, Boguta M. Maf1 is involved in coupling carbon metabolism to RNA polymerase III transcription. *Mol Cell Biol*. 2007; 27:7693–7702. [PubMed: 17785443]
18. Kolesnikova OA, Entelis NS, Mireau H, Fox TD, Martin RP, Tarassov IA. Suppression of mutations in mitochondrial DNA by tRNAs imported from the cytoplasm. *Science*. 2000; 289:1931–1933. [PubMed: 10988073]
19. Rinehart J, Krett B, Rubio MAT, Alfonzo JD, Söll D. *Saccharomyces cerevisiae* imports the cytosolic pathway for Gln-tRNA synthesis into the mitochondrion. *Genes Dev*. 2005; 19:583–592. [PubMed: 15706032]
20. Arimbasseri AG, Blewett NH, Iben JR, Lamichhane TN, Cherkasova V, Hafner M, Maraia RJ. RNA polymerase III output is functionally linked to tRNA dimethyl-G26 modification. *PLoS Genet*. 2015; 11:e1005671. [PubMed: 26720005]
21. Kwapisz M, Smagowicz WJ, Oficjalska D, Hatin I, Rousset JP, Zoladek T, Boguta M. Up-regulation of tRNA biosynthesis affects translational readthrough in maf1-delta mutant of *Saccharomyces cerevisiae*. *Curr Genet*. 2002; 42:147–152. [PubMed: 12491008]
22. Morawiec E, Wichtowska D, Graczyk D, Conesa C, Lefebvre O, Boguta M. Maf1, repressor of tRNA transcription, is involved in the control of gluconeogenic genes in *Saccharomyces cerevisiae*. *Gene*. 2013; 526:16–22. [PubMed: 23657116]
23. Rønnow B, Kielland-Brandt MC. GUT2, a gene for mitochondrial glycerol 3-phosphate dehydrogenase of *Saccharomyces cerevisiae*. *Yeast*. 1993; 9:1121–1130. [PubMed: 8256521]
24. Steinmetz LM, Scharfe C, Deutschbauer AM, Mokranjac D, Herman ZS, Jones T, Chu AM, Giaever G, Prokisch H, Oefner PJ, Davis RW. Systematic screen for human disease genes in yeast. *Nat Genet*. 2002; 31:400–404. [PubMed: 12134146]
25. Johnson SS, Zhang C, Fromm J, Willis IM, Johnson DL. Mammalian Maf1 is a negative regulator of transcription by all three nuclear RNA polymerases. *Mol Cell*. 2007; 26:367–379. [PubMed: 17499043]
26. Palian BM, Rohira AD, Johnson SAS, He L, Zheng N, Dubeau L, Stiles BL, Johnson DL. Maf1 is a novel target of PTEN and PI3K signaling that negatively regulates oncogenesis and lipid metabolism. *PLoS Genet*. 2014; 10:e1004789. [PubMed: 25502566]
27. Khanna A, Johnson DL, Curran SP. Physiological roles for maf1-1 in reproduction and lipid homeostasis. *Cell Rep*. 2014; 9:2180–2191. [PubMed: 25497095]
28. Li Y, Tsang CK, Wang S, Li XX, Yang Y, Fu L, Huang W, Li M, Wang HY, Zheng XFS. MAF1 suppresses AKT-mTOR signaling and liver cancer through activation of PTEN transcription. *Hepatology*. 2016; 63:1928–1942. [PubMed: 26910647]
29. Cieła M, Mierzejewska J, Adamczyk M, Farrants A-KÖ, Boguta M. Fructose bisphosphate aldolase is involved in the control of RNA polymerase III-directed transcription. *Biochim Biophys Acta*. 2014; 1843:1103–1110. [PubMed: 24576411]

30. Hoffmann NA, Jakobi AJ, Vorländer MK, Sachse C, Müller CW. Transcribing RNA polymerase III observed by electron cryomicroscopy. *FEBS J.* 2016; 283:2811–2819. [PubMed: 27059519]
31. Cie la M, Makala E, Plonka M, Bazan R, Gewartowski K, Dziembowski A, Boguta M. Rbs1, a new protein implicated in RNA polymerase III biogenesis in yeast *Saccharomyces cerevisiae*. *Mol Cell Biol.* 2015; 35:1169–1181. [PubMed: 25605335]
32. Collins SR, Kemmeren P, Zhao XC, Greenblatt JF, Spencer F, Holstege FCP, Weissman JS, Krogan NJ. Toward a comprehensive atlas of the physical interactome of *Saccharomyces cerevisiae*. *Mol Cell Proteomics.* 2007; 6:439–450. [PubMed: 17200106]
33. Berg, JM., Tymoczko, JL., Gatto, GJ., Stryer, L. Chapter 16 glycolysis and gluco-neogenesis, *Biochemistry*. Eighth. Freeman, WH., editor. Company, a Macmillan Education Imprint; New York: 2015.
34. Peeters K, Van Leemputte F, Fischer B, Bonini BM, Quezada H, Tsytlonok M, Haesen D, Vanthienen W, Bernardes N, Gonzalez-Blas CB, Janssens V, Tompa P, Versées W, Thevelein JM. Fructose-1,6-bisphosphate couples glycolytic flux to activation of Ras. *Nat Commun.* 2017; 8:922. [PubMed: 29030545]
35. Zhang CS, Hawley SA, Zong Y, Li M, Wang Z, Gray A, Ma T, Cui J, Feng JW, Zhu M, Wu YQ, Li TY, Ye Z, Lin SY, Yin H, Piao HL, Hardie DG, Lin SC. Fructose-1,6-bisphosphate and aldolase mediate glucose sensing by AMPK. *Nature.* 2017; 548:112–116. [PubMed: 28723898]
36. Rideout EJ, Marshall L, Grewal SS. Drosophila RNA polymerase III repressor Maf1 controls body size and developmental timing by modulating tRNAiMet synthesis and systemic insulin signaling. *Proc Natl Acad Sci U S A.* 2012; 109:1139–1144. [PubMed: 22228302]
37. Marshall L, Rideout EJ, Grewal SS. Nutrient/TOR-dependent regulation of RNA polymerase III controls tissue and organismal growth in *Drosophila*. *EMBO J.* 2012; 31:1916–1930. [PubMed: 22367393]
38. Grewal SS. Why should cancer biologists care about tRNAs? tRNA synthesis, mRNA translation and the control of growth. *Biochim Biophys Acta.* 2015; 1849:898–907. [PubMed: 25497380]
39. Owusu-Ansah E, Perrimon N. Stress signaling between organs in metazoa. *Annu Rev Cell Dev Biol.* 2015; 31:497–522. [PubMed: 26393775]
40. Orioli A, Praz V, Lhôte P, Hernandez N. Human MAF1 targets and represses active RNA polymerase III genes by preventing recruitment rather than inducing long-term transcriptional arrest. *Genome Res.* 2016; 26:624–635. [PubMed: 26941251]
41. Turowski TW, Tollervey D. Transcription by RNA polymerase III: insights into mechanism and regulation. *Biochem Soc Trans.* 2016; 44:1367–1375. [PubMed: 27911719]
42. Cai Y, Wei YH. Stress resistance and lifespan are increased in *C. elegans* but decreased in *S. cerevisiae* by *mafr-1/maf1* deletion. *Oncotarget.* 2016; 7:10812–10826. [PubMed: 26934328]
43. Antikainen H, Driscoll M, Haspel G, Dobrowolski R. TOR-mediated regulation of metabolism in aging. *Aging Cell.* 2017; 16:1219–1233. 16. [PubMed: 28971552]
44. Powers RW, Kaerberlein M, Caldwell SD, Kennedy BK, Fields S. Extension of chronological life span in yeast by decreased TOR pathway signaling. *Genes Dev.* 2006; 20:174–184. [PubMed: 16418483]
45. Pradhan A, Hammerquist AM, Khanna A, Curran SP. The C-Box region of MAF1 regulates transcriptional activity and protein stability. *J Mol Biol.* 2017; 429:192–207. [PubMed: 27986570]
46. Vannini A, Ringel R, Kusser AG, Berninghausen O, Kassavetis GA, Cramer P. Molecular basis of RNA polymerase III transcription repression by Maf1. *Cell.* 2010; 143:59–70. [PubMed: 20887893]
47. Bonhoure N, Byrnes A, Moir RD, Hodroj W, Preitner F, Praz V, Marcelin G, Chua SC, Martinez-Lopez N, Singh R, Moullan N, Auwerx J, Willemin G, Shah H, Hartil K, Vaitheeswaran B, Kurland I, Hernandez N, Willis IM. Loss of the RNA polymerase III repressor MAF1 confers obesity resistance. *Genes Dev.* 2015; 29:934–947. [PubMed: 25934505]
48. Reina JH, Azzouz TN, Hernandez N. Maf1, a new player in the regulation of human RNA polymerase III transcription. *PLoS One.* 2006; 1:e134. [PubMed: 17205138]
49. Johnson SAS, Dubeau L, Kawalek M, Dervan A, Schöenthal AH, Dang CV, Johnson DL. Increased expression of TATA-binding protein, the central transcription factor, can contribute to oncogenesis. *Mol Cell Biol.* 2003; 23:3043–3051. [PubMed: 12697807]

50. Quin JE, Devlin JR, Cameron D, Hannan KM, Pearson RB, Hannan RD. Targeting the nucleolus for cancer intervention. *Biochim Biophys Acta*. 2014; 1842:802–816. [PubMed: 24389329]
51. Johnson SAS, Dubeau L, Johnson DL. Enhanced RNA polymerase III-dependent transcription is required for oncogenic transformation. *J Biol Chem*. 2008; 283:19184–19191. [PubMed: 18456653]
52. White RJ. RNA polymerases I and III, non-coding RNAs and cancer. *Trends Genet*. 2008; 24:622–629. [PubMed: 18980784]
53. Johnson DL, Stiles BL. Maf1, A new PTEN target linking RNA and lipid metabolism, *Trends Endocrinol. Metab*. 2016; 27:742–750.
54. Tokheim CJ, Papadopoulos N, Kinzler KW, Vogelstein B, Karchin R. Evaluating the evaluation of cancer driver genes. *Proc Natl Acad Sci U S A*. 2016; 113:14330–14335. [PubMed: 27911828]
55. Forbes SA, Beare D, Boutselakis H, Bamford S, Bindal N, Tate J, Cole CG, Ward S, Dawson E, Ponting L, Stefancsik R, Harsha B, Kok CY, Jia M, Jubb H, Sondka Z, Thompson S, De T, Campbell PJ. COSMIC: somatic cancer genetics at high-resolution. *Nucleic Acids Res*. 2017; 45:D777–D783. [PubMed: 27899578]
56. Frühbeck G, Méndez-Giménez L, Fernández-Formoso JA, Fernández S, Rodríguez A. Regulation of adipocyte lipolysis. *Nutr Res Rev*. 2014; 27:63–93. [PubMed: 24872083]
57. Eisenberg T, Knauer H, Schauer A, Büttner S, Ruckstuhl C, Carmona-Gutierrez D, Ring J, Schroeder S, Magnes C, Antonacci L, Fussi H, Deszcz L, Hartl R, Schraml E, Criollo A, Megalou E, Weiskopf D, Laun P, Heeren G, Breitenbach M, et al. Induction of autophagy by spermidine promotes longevity. *Nat Cell Biol*. 2009; 11:1305–1314. [PubMed: 19801973]
58. Wang W, Seale P. Control of brown and beige fat development. *Nat Rev Mol Cell Biol*. 2016; 17:691–702. [PubMed: 27552974]
59. Bonhoure N, Bounova G, Bernasconi D, Praz V, Lammers F, Canella D, Willis IM, Herr W, Hernandez N, Delorenzi M. CycliX Consortium, Quantifying ChIP-seq data: a spiking method providing an internal reference for sample-to-sample normalization. *Genome Res*. 2014; 24:1157–1168. [PubMed: 24709819]
60. Hanahan D, Weinberg RA. Hallmarks of cancer: the next generation. *Cell*. 2011; 144:646–674. [PubMed: 21376230]
61. Altman BJ, Stine ZE, Dang CV. From Krebs to clinic: glutamine metabolism to cancer therapy. *Nat Rev Cancer*. 2016; 16:619–634. [PubMed: 27492215]
62. Davidson SM, Papagiannakopoulos T, Olenchok BA, Heyman JE, Keibler MA, Luengo A, Bauer MR, Jha AK, O'Brien JP, Pierce KA, Gui DY, Sullivan LB, Wasylenko TM, Subbaraj L, Chin CR, Stephanopoulos G, Mott BT, Jacks T, Clish CB, Vander Heiden MG. Environment impacts the metabolic dependencies of Ras-driven non-small cell lung cancer. *Cell Metab*. 2016; 23:517–528. [PubMed: 26853747]
63. Muir A, Danai LV, Gui DY, Waingarten CY, Lewis CA, Vander Heiden MG. Environmental cystine drives glutamine anaplerosis and sensitizes cancer cells to glutaminase inhibition, *elife* 6 (2017).
64. Lee YL, Li YC, Su CH, Chiao CH, Lin IH, Hsu MT. MAF1 represses CDKN1A through a Pol III-dependent mechanism. *elife*. 2015; 4:e06283. [PubMed: 26067234]
65. Yeganeh M, Praz V, Cousin P, Hernandez N. Transcriptional interference by RNA polymerase III affects expression of the Polr3e gene. *Genes Dev*. 2017; 31:413–421. [PubMed: 28289142]
66. Van Bortle K, Phanstiel DH, Snyder MP. Topological organization and dynamic regulation of human tRNA genes during macrophage differentiation. *Genome Biol*. 2017; 18:180. [PubMed: 28931413]

Fig. 1. Metabolic enzymes linked to Pol III and Maf1 in yeast. **A** Entry of glycerol into the glycolytic/gluconeogenic pathway. Double headed arrows indicate reversible reactions. Pairs of arrows indicate irreversible steps for which separate enzymes are required. Gluconeogenic enzymes discussed in the text are shown in red, aldolase is shown in green. The red asterisks highlight the positive allosteric regulation of pyruvate kinase by fructose 1,6, biphosphate. Key functions of sugars in the upper part of the pathway are indicated as discussed in the text. **B** Top view of the Pol III elongating complex [30]. The boxed region, enlarged below, shows the position of the *rpc128-1007* mutation in green and residues containing atoms within 5 Å. These include atoms from three amino acids at the interface with AC40 in magenta and Rpc128 amino acids spanning residues 1005–1010 and 929–933 in dark blue. **C** A hypothetical scheme suggests that when glucose and F-1,6-P levels are high, binding of the latter to Fba1 (aldolase) in complex with Pol III may promote transcription.

Fig. 2.

Schematic model of metabolic inefficiency in *Maf1* KO mice. The central hypothesis is that loss of *Maf1* in all tissues results in increased synthesis and turnover of Pol III transcripts, most notably tRNAs, throughout the diurnal cycle. This increases the demand for cellular energy (ATP) in order to maintain nucleotide concentrations since these are being consumed at a greater rate by Pol III. This, in turn, drives metabolic changes in various tissues, as indicated, to provide substrates for oxidative metabolism.

Table 1

Maf1 phenotypes in model organisms and cell-based systems.

Organism or cell-based model	Phenotype	Perturbation	Refs.
<i>D. melanogaster</i>	Increased larval growth rate and adult body size ^a	<i>dMaf1</i> RNAi, ubiquitous and fat body-specific expression or an extra copy of tRNA _i ^{Met}	[36]
	Increased protein synthesis.	Extra copy of tRNA _i ^{Met}	
	Increased systemic insulin signaling (non-cell-autonomous phenotype).	Fat body-specific <i>dMaf1</i> RNAi or an extra copy of tRNA _i ^{Met}	
<i>C. elegans</i>	Fecundity decreased ^b	<i>mafr-1</i> overexpression (single extra copy)	[27]
	Expression of vitellogenin genes and many other protein coding genes decreased.		
	Expression of TBP, <i>fasn-1</i> and <i>pod-2</i> (aka Acc1) decreased.		
	Reduced fat accumulation ^b		
	<i>thp-1</i> , <i>fasn-1</i> and <i>pod-2</i> expression increased.	<i>mafr-1</i> RNAi	
	Increased intestinal fat.		
	Induced stress response and mitochondrial unfolded protein response.	<i>mafr-1(tm6082)</i>	[42]
	Autophagy increased ^c		
	Lifespan increased ^c		
Glioblastoma cells	Lifespan increased ^c	<i>mafr-1</i> RNAi	[25]
	Pol I transcription increased and decreased.	<i>Maf1</i> shRNA and overexpression	
	TBP, Egr-1 expression increased and decreased.		
Transformed murine hepatocytes or human hepatocarcinoma cells	Decreased anchorage-independent growth.	<i>Maf1</i> overexpression	[26,28]
	Decreased growth, proliferation and invasiveness of tumor cells	<i>Maf1</i> overexpression	
	Increased growth, proliferation and invasiveness of tumor cells	<i>Maf1</i> shRNA	[28]
	PTEN expression increased and decreased	<i>Maf1</i> overexpression and shRNA	
	<i>Fasn</i> and <i>Acc1</i> expression increased and decreased.	<i>Maf1</i> shRNA and overexpression	[26]
	Increased intracellular lipids ^a	<i>Maf1</i> shRNA	
Mouse xenograft	Decreased rate of tumor growth.	<i>Maf1</i> overexpression	[26,28]
Mouse liver	Decreased triglycerides ^b	<i>Maf1</i> overexpression from adenovirus vector.	[26]
	Decreased triglycerides	<i>Maf1</i> KO	
	Increased autophagy and lipophagy		[47]
	Increased fatty acid synthesis		
	Increased amino acids and polyamines		
	Reduced long chain acyl carnitines		
	Global reduction in protein synthesis		
	Increased lipolysis		
Mouse WAT			

Organism or cell-based model	Phenotype	Perturbation	Refs.
Mouse skeletal muscle	Increased amino acids and polyamines Increased NAD ⁺		
Mouse	Decreased fertility and fecundity Lean body composition, obesity resistant		

^aOpposite phenotype seen in *Maf1* KO mice.

^bSame phenotype caused by the opposite perturbation of Maf1 in the mouse.

^cSame phenotype, due to recessive loss of function mutation, as the *Maf1* KO mouse.