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Human *TYRP1*: two functions for a single gene?

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SUMMARY

In the animal kingdom, skin pigmentation is highly variable between species, and it contributes to phenotypes. In humans, skin pigmentation plays a part in sun protection. Skin pigmentation depends on the ratio of the two pigments pheomelanin and eumelanin, both synthesized by a specialized cell population, the melanocytes. In this review, we explore one important factor in pigmentation: the *tyrosinase-related protein 1* (*TYRP1*) gene which is involved in eumelanin synthesis via the TYRP1 protein. Counterintuitively, high *TYRP1* mRNA expression is associated with a poor clinical outcome for patients with metastatic melanomas. Recently, we were able to explain this unexpected *TYRP1* function by demonstrating that *TYRP1* mRNA sequesters microRNA-16, a tumour suppressor miRNA. Here, we focus on actors influencing *TYRP1* mRNA abundance, particularly transcription factors, single-nucleotide polymorphisms (SNPs), and miRNAs, as they all dictate the indirect oncogenic activity of *TYRP1*.

KEYWORDS

melanoma, microRNA, TYRP1, SNP, miRNA sponge, pigmentation

INTRODUCTION

Over the last decade, targeted therapies and immune checkpoint inhibitors have significantly improved the management of patients with melanomas. The choice of treatment is based both on the detection of driver mutations and on anatomopathological analysis that explores histopathological criteria and the immunohistochemical characteristics of the melanoma (Ascierto et al., 2020). These investigations define the percentage of tumor cells per sample, and are based on the detection of highly expressed markers that are found specifically in melanocytes, including human melanoma black-45 (HMB-45), Melan-A, tyrosinase (TYR), S100, and TYRP1 (Gogas et al., 2009). These biomarkers can also be used to characterize tumor heterogeneity during relapse (Bai, Fisher, & Flaherty, 2019; Rambow et al., 2018). Recently, we and other researchers showed that *TYRP1* mRNA and proteins are more than just humble markers (El Hajj, Gilot, Migault, Theunis, Van Kempen, et al., 2015; El Hajj et al., 2013; Ghanem & Journe, 2011; Gilot et al., 2017). Therefore, an in-depth knowledge of the regulation of *TYRP1* promoter, RNAs, and proteins is important to elucidate the “indirect oncogenic activity” of *TYRP1* mRNA, which is based on miR-16 sequestration. Moreover, the restricted expression of *TYRP1* in melanocytes and the retinal pigment epithelium (RPE) suggests that *TYRP1* mRNA might be a remarkable target for cancer therapy, as there is no fear of harmful effects on the other cells in the body, which are all *TYRP1*-negative. We also discuss here an antisense oligonucleotide strategy aimed at avoiding miRNA sequestration on *TYRP1*, thereby restoring the mRNA activity of the tumor suppressor miR-16.

***TYRP1* GENE REGULATION**

▪ Gene

Human *TYRP1* cDNA was isolated from melanoma cells in 1990. This gene is located on chromosome 9 (9p23) at base pairs 12,693,385 to 12,710,266 in the NCBI GRCh38/hg38 assembly. It encodes the human homolog of the mouse *brown (b)* locus gene product (Bennett, Huszar, Laipis, Jaenisch, & Jackson, 1990; Jackson, 1988). The human *TYRP1* gene is spread over 24 kbp of genomic DNA, as compared to 18 kbp for the mouse version (Shibahara, Tomita, Yoshizawa, Shibata, & Tagami, 1992; Sturm et al., 1995), and the human TYRP1 protein is encoded by 7 exons while the mouse version is encoded by 8 (Figure 1).

The *TYRP1* GeneID at NCBI is 7306 (<https://www.ncbi.nlm.nih.gov/gene/7306>). Additional information is available on other web sites, including [Ensembl:ENSG00000107165](#), [MIM:115501](#), and [Vega:OTTHUMG00000021034](#). Synonyms for *TYRP1* include *OCA3*, *TYRRP*, *GP75*, *CATB*, *TRP*, *b-PROTEIN*, *TYRP*, *CAS2*, and *TRP1*.

▪ Expression

TYRP1 is involved in the production of melanin pigment, so it is mostly expressed in cell types that produce melanin, including melanocytes and the RPE (Murisier & Beerman, 2006). The RPE originates from the optic neuroepithelium, is located close to the retina, and is crucial for eye organogenesis and vision (Bharti, Nguyen, Skuntz, Bertuzzi, & Arnheiter, 2006). Melanocytes, which are derived from the neural crest, can be classified into two groups: cutaneous/classical melanocytes which can be found in the skin (epidermis and dermis); and non-cutaneous/non-classical melanocytes which colonize the eye, inner ear, meninges, heart, and adipose tissues (Petit & Larue, 2016; Randhawa et al., 2009; Yajima & Larue, 2008). While *TYRP1* can be detected in tissues colonized by non-cutaneous melanocytes, this RNA is mainly detected in the skin (Figure 2a).

Most cutaneous melanocytes are located in the epidermis, and are follicular and interfollicular (often called “epidermal”) melanocytes. These are both involved in hair and skin pigmentation as well as in protecting skin against DNA damage or oxidative stress. Melanocytes in the hair follicles also contribute to the elimination of the toxic by-products that result from melanin synthesis, while epidermal melanocytes are involved in inflammatory response by acting as phagocytic cells (Colombo, Berlin, Delmas, & Larue, 2011). *TYRP1* expression in both cell types is dependent on melanocyte differentiation, and seems to be necessary for the maturation of the melanosome, the organelle that synthesizes, stores, and transports melanin. Only melanocytes with mature melanosomes (types III and IV) seem to express *TYRP1* (Cichorek, Wachulska, Stasiewicz, & Tymińska, 2013; Jimbow et al., 2000; Raposo & Marks, 2007).

Follicular melanocyte maturation follows the hair development cycle, starting with the anagen growth phase of active melanogenesis, then a regressive phase where mature melanocytes undergo apoptosis, and finally the telogen quiescent phase (Qiu, Chuong, & Lei, 2019; Schneider, Schmidt-Ullrich, & Paus, 2009). During the hair cycle, *TYRP1* is only expressed in the anagenic follicular melanocytes localized in the hair matrix which are responsible for hair pigmentation (Slominski et al., 2005). Both hair cycles and pigmentation are regulated by the circadian clock, and *TYRP1* levels have been shown to depend on the expression of clock genes. Indeed, silencing the core clock genes *BMAL1* and *PER1* extends the anagen phase and increases *TYRP1* expression levels (Hardman et al., 2015; Plikus et al., 2015).

TYRP1 is also highly expressed in tumors derived from melanocytes, cutaneous and uveal melanomas. In benign nevus and melanoma tumors, *TYRP1* mRNA expression levels are variable (Figures 2b and 2c). The three members of the tyrosinase family, tyrosinase (*TYR*), the dopachrome tautomerase (*DCT/TYRP2*), and *TYRP1*, are not strictly correlated even if their expression levels are all at least in part governed by the same transcription factor, MITF (Melanocyte/Microphthalmia-associated transcription factor). In melanoma biopsies from the cutaneous skin cancer cohort in the Cancer Genome

Atlas (TCGA), the expression pattern of *TYRP1* is well correlated with those of *TYR* and *MLANA* (Figure 2c). *TYRP1* can also be detected in other types of cancers arising from non-melanocytic lineage tissues such as colon and breast cancers (Hsu et al., 2018; Montel, Suzuki, Galloy, Mose, & Tarin, 2009). Taken together, these studies highlight the specific *TYRP1* expression profile in tissues, as well as its involvement in the pigmentation process.

▪ *TYRP1* gene promoter and enhancer

TYRP1 expression is tightly associated with melanocyte differentiation and pigmentation. Even if MITF plays a predominant role in *TYRP1* expression by targeting the proximal promoter, *TYRP1* expression also depends on other transcriptional factors as well as on a distal enhancer.

• Role of the distal enhancer of *Tyrp1*

Numerous positive and negative transcription regulators have been identified for the *TYRP1* promoter (Table 1 and Figure 3). Data have been mainly produced from murine models (*Tyrp1*). MITF's ability to transactivate *TYRP1* and *Tyrp1* promoters has been clearly demonstrated in numerous studies including those in knockout mice (Hemesath et al., 1994; Hodgkinson et al., 1993). MITF belongs to the MITF/TFE group in the basic helix-loop-helix—leucine-zipper (bHLH-LZ) transcription factor family (Hemesath et al., 1994; Hodgkinson et al., 1993; Nakayama et al., 1998). The bHLH-LZ transcription factors bind an E-box consensus sequence (CANNTG), while MITF binds the M-box, a sequence of 11 bp that contains an E-box core (AGTCATGTGCT). In mice, this box is located upstream of the TATA box in the *Tyrp1* promoter and transactivates it (Bertolotto et al., 1998). Furthermore, even if it is to a lesser extent, Mitf induces *Tyrp1* expression by binding an E-box (CAAGTG) located at -238/-233 (Bertolotto et al., 1998). In human cells, data from chromatin immunoprecipitation (ChIP) experiments followed by DNA sequencing have demonstrated a physical interaction between MITF and *TYRP1* (Strub et al., 2011). Moreover, *MITF* cDNA overexpression promotes *TYRP1* expression by causing MITF to bind to the M-box (Yasumoto, Yokoyama, Takahashi, Tomita, & Shibahara, 1997). The human *TYRP1* M-box (AATCATGTGCT) occurs from -197 to -186 on the GCRh38/hg38 assembly, and it is a strong activator of pigment cells (Shibata, Takeda, Tomita, Tagami, & Shibahara, 1992; Yasumoto et al., 1997).

While both MITF and the M-box are required for *TYRP1* expression in melanocytes, a conserved transcriptional enhancer also seems to play a crucial role (Marathe et al., 2017; Murisier, Guichard, & Beermann, 2006). This element is located at approximately -15 kb and contains Sox10 binding sites, which are perfectly conserved between the mouse and human genomes. By combining ChIP experiments and formaldehyde-assisted isolation of regulatory elements (FAIRE) analysis, the authors showed that the distal enhancers of *Tyrp1* are in an inherently open chromatin state prior to

differentiation. Upon differentiation of mouse melanoblast Melb-a cells, the chromatin becomes accessible at the proximal promoter of *Tyrp1* (Marathe et al., 2017). This study strongly suggests that SOX10 binds to the distal enhancer and recruits brahma-related gene-1 (BRG1) to form a loop with the proximal promoter, which contains MITF-binding sites. In Figure 3b, we present a schema illustrating this hypothetical mechanism. A chromatin loop promoting the connection between enhancer and proximal promoter of *Tyrp1*, likely occurs in the presence of the three main actors, SOX10, BRG1, and MITF. MITF transcription factor expression increases during differentiation, and its rising expression allows for the transactivation of pigmentation genes such as *Tyrp1* and *Tyr*.

- Role of the first intron in *Tyrp1* mRNA expression

The mouse *Tyrp1* promoter contains MSEu and MSEi, two related *cis*-acting elements that are upstream and initiator melanocyte-specific elements, respectively. These two GTGTGA sequences are binding sites for Pax3 and Tbx2 (Carreira, Dexter, Yavuzer, Easty, & Goding, 1998; Galibert, Yavuzer, Dexter, & Goding, 1999). Pax3 binding on MSEu (-241 to -235 in the GRCm38/mm10 assembly) and on MSEi leads to *Tyrp1* transcription activation, whereas Tbx2 acts as a repressor (Carreira et al., 1998). These two sites and their respective positions are not conserved in the human *TYRP1* promoter. Two GTGTGA sequences separated by 21 nucleotides are found in the *TYRP1* intron 1, (+334 to +340 and +361 to +367 in the GRCh38/hg38 assembly). Moreover, it has been proposed that *TYRP1* is regulated by PAX3 through a TGTCACACTT sequence (Corry & Underhill, 2005). In human *TYRP1*, this sequence is localized in intron 1 between the two GTGTGA sequences just discussed. This finding is of particular interest because it is possible that the *TYRP1* exon 1 and intron 1 are important for *TYRP1* regulation (Murisier et al., 2006).

- Transcription factors E3 and EB (Tfe3 and Tfeb)

MITF is not the only bHLH-LZ transcription factor able to promote *TYRP1* expression. Tfeb and Tfe3 share a high homology with Mitf, and in the B16 mouse melanoma cell line their ectopic expression transactivates the *Tyrp1* promoter by binding the M-box (Verastegui et al., 2000). On the other hand, endogenous Tfe3 cannot bind to the M-box as a homodimer, and the authors did not detect any Tfe3/Mitf heterodimers. It is therefore difficult to be sure of the physiological roles of Tfe3 and Tfeb in basal *Tyrp1* expression when Mitf proteins are present, and further investigations are required to prove their ability to promote *Tyrp1* without ectopic expression. As observed in Mitf-knockout mice, there is a strong decrease in *Tyrp1* levels when Mitf is depleted (Hemesath et al., 1994; Hodgkinson et al., 1993). This suggests that Mitf is required for *Tyrp1* induction, and Tfe3 and/or Tfeb are not able to compensate for the lack of Mitf and induce *Tyrp1*.

- Inducibility of *TYRP1* gene by ultraviolet (UV) radiation

Interestingly, p53, which is activated in response to UV rays, can bind and transactivate the *TYRP1* promoter via two clusters, each of which is composed of three binding sites (Nylander et al., 2000). These clusters are separated by 14 nucleotides, and their binding sites are all located in the -121 to -42 fragment. The p53-homolog proteins p63 α and p73 α also transactivate the *TYRP1* promoter.

- *Tyrp1* expression repressors

In normal cells, *TYRP1* mRNA is mainly expressed in melanocytes and in the RPE (Figure 2). The orthodenticle homeobox 2 (OTX2) protein transactivates the *TYRP1* promoter via three binding sites (Martínez-Morales et al., 2003). OTX2 is an RPE-specific factor, while SOX10 and PAX3 are specifically expressed in melanocytes (Murisier & Beermann, 2006) and in testes. These transcription factors are also at least partly responsible for *TYRP1* cell-specific expression.

In conclusion, the transcription factors governing murine *Tyrp1* expression are better known than those driving human *TYRP1*. In order to map the regulatory elements of the human *TYRP1* gene, it might be interesting to perform *in situ* tiling screens, using clustered regularly interspaced short palindromic repeats (CRISPR) for saturation mutagenesis experiments along the *TYRP1* promoter (Canver et al., 2015).

- RNA

The reference sequences (RefSeqs) for *TYRP1* mRNA is NM_000550.3 in NCBI and ENST00000388918.9 in Ensembl (Figure 1). The mRNA length is 2,896 bp. The gene has 7 transcripts (splice variants), 346 orthologs, and 2 paralogs. It is a member of one Ensembl protein family and is associated with one phenotype (Sarangarajan & Boissy, 2001): Type 3 oculocutaneous albinism (OCA3), described in more detail below.

The NM_000550.3 transcript contains 8 exons and is associated with 526 variations. Importantly, NM_000550.3 replaced NM_000550.2 in 2019 as the official RefSeq (Figure 4). NM_000550.3 and NM_000550.2 differ by 1 bp in the 5' untranslated region (UTR) and 19 bp in the 3'-UTR. In contrast, NM_000550.1 has two deletions of 4 nucleotides and four single nucleotide polymorphisms (SNPs) in the 3'-UTR, as well as being shorter (99 bp) than NM_000550.3. To date, the consequence of the two deletions observed in NM_000550.1 (AAGT, 2090-2094 and ATTA, 2264-2268) remains elusive. Interestingly, the chimpanzee genome also contains these two deletions, which suggests that the differences between NM_000550.1 and NM_000550.3 are probably not the result of sequencing experiment artifacts.

The four SNPs that differ between the NM_000550.3 and NM_000550.1 transcripts are rs683, rs2762464, rs910, and rs1063380 (Figure 4). Due to their physical proximity, these SNPs are tightly linked and inherited together (Jingjing Li et al., 2012). These SNP alleles are associated with individuals from various backgrounds, including Caucasians (CEU CEPH, Utah Residents with Northern and Western European Ancestry), Asians (JPT, Japanese in Tokyo, Japan), and Africans (YRI, Yoruba in Ibadan, Nigeria). In 2012, Jingjing Li and colleagues demonstrated that rs683 and rs910 determine *TYRP1* mRNA levels (Li et al., 2012), and they also showed that these SNPs are located in the microRNA response elements (MREs) for miR-155 (Figure 5).

MicroRNAs (miRNAs) are small (~22 nt) noncoding RNAs that mediate mRNA translation arrest and/or RNA decay through a perfect base pairing between miRNA seed sequences (nt 2-7) and the MRE of their RNA targets (Bartel, 2018a). Three MRE-155s have been identified on the *TYRP1* 3'-UTR (Figure 5a). We recently confirmed that the first one seems to play a more minor role in *TYRP1* RNA decay than to the other two (Gilot et al., 2017), and this is probably due to the proximity of the stop codon and the ribosome passage on this region. We also showed that the rs910 SNP in the third MRE-155 exerts a moderately increased effect on *TYRP1* expression over that of rs683 in the second MRE. Indeed, the rs683-A allele, *TYRP1*-A from NM_000550.1, reduces miR-155-induced *TYRP1* mRNA decay much more strongly than the C allele, *TYRP1*-C from NM_000550.3 (El Hajj, Gilot, Migault, Theunis, van Kempen, et al., 2015; Jingjing Li et al., 2012). Our results confirm that miR-155 affects *TYRP1*-A mRNA less than it affects *TYRP1*-C mRNA. In other words, the *TYRP1* mRNAs expressed by two-thirds of CEU individuals carry the ancestral allele which disrupts the interaction between miR-155 and *TYRP1*, thereby contributing to elevated levels of *TYRP1* mRNA in CEU individuals. In contrast, miR-155 strongly reduces the half-lives of the *TYRP1*-C alleles in the JPT and YRI cohorts (Figures 5b and c).

Interestingly, Jingjing Li et al. analyzed the expression levels of *TYRP1* as a function of population localization across the globe. They observed a strong negative correlation between the population's latitude of residence and the miR-155-mediated repression of *TYRP1* (Jingjing Li et al., 2012). Thus, people with high levels of sun exposure may display a faster turnover of *TYRP1* than other populations.

Besides SNPs, other factors such as RNA-binding protein (RBPs) have been described as modulating the binding of miRNA on target sequences (Soemedi, Vega, Belmont, Ramachandran, & Fairbrother, 2014). Reciprocally, SNPs located on miRNA genes may affect mature sequences of the miRNAs and consequently modify their MRE-binding affinity. Huang et al. showed that miR-196a-2 carrying the rs11614913 C allele downregulates *TYRP1* expression more strongly than the miRNA with the T allele. They proposed that this model could explain the relatively low *TYRP1* expression in normal human melanocytes that contain that rs11614913 C allele (Huang et al., 2013). The authors also showed that this miR-196a-2 C-variant genotype is associated with a decreased risk of vitiligo, a depigmentation disorder characterized by the destruction of melanocytes because of an inherent sensitivity to oxidative

stress (Cui et al., 2015; Jimbow, Chen, Park, & Thomas, 2001; Picardo et al., 2015). The researchers hypothesized that this miR-196a-2 variant downregulates its targets, including *TYRP1*, thereby exerting potentially protective effects on human melanocytes under oxidative stress and reducing the risk of vitiligo (Huang et al., 2013). It has been proposed that normal TYRP1 protein expression protects vitiligo melanocytes from early death (Jimbow et al., 2001; Mansuri, Singh, & Begum, 2016), indicating that miRNA regulation in the expression of *TYRP1* in vitiligo should be clarified.

We recently identified three miR-16 binding sites on the *TYRP1* 3'-UTR (Figure 5a). These sites are common on the two alleles (NM_000550.3 and NM_000550.1). These MRE-16s have a noncoding role in *TYRP1* mRNA function, and this is discussed at the end of this review.

▪ **TYRP1 protein and its functions**

TYRP1 mRNA encodes TYRP1, a protein containing 537 amino acids (NP_000541.1 or ENSP00000373570). TYRP1 is synthesized as a core 55 kDa polypeptide (Figures 1 and 6), and the mature glycoprotein is ~75 kDa (Vijayasradhi, Doskoch, & Houghton, 1991). Mature TYRP1 is a transmembrane protein that contains two zinc ions in its active sites instead of the copper ions found in tyrosinase (Lai, Wichers, Soler-Lopez, & Dijkstra, 2017, 2018). According to Ensembl (Figure 1), different TYRP1 proteins can be translated from three transcripts (splice variants). Despite using three different anti-TYRP1 antibodies that target different epitopes (G-17, Ab23, and PEP1), we were not able to identify the other putative TYRP1 proteins in cutaneous melanoma biopsies (Gilot et al., 2017).

Human and mouse TYRP1/Typr1 have six potential *N*-glycosylation sites (Figure 6) (Vijayasradhi et al., 1991; Xu et al., 2001). The mean *N*-glycan composition of Typr1 is 16% high mannose, 16% biantennary, and more than 65% tri- and tetra-antennary structures (Branza-Nichita, Petrescu, Negroiu, Dwek, & Petrescu, 2000; Negroiu, Branza-Nichita, Petrescu, Dwek, & Petrescu, 1999). This pattern of glycosylation seems important for TYRP1 processing, trafficking, and stability (Xu et al., 2001). The role of the *N*-glycosylation of TYR family members was recently discussed by Lai et al. (Lai, Wichers, Soler-Lopez, & Dijkstra, 2020), who identified Asn371 on TYR as the most important *N*-glycosylation site for enzyme functioning. It might improve TYR stability and/or activity, and it corresponds to amino acid 385 in the human TYRP1 protein. Based on the crystal structure of glycosylated human TYRP1, the authors suggested that Asn385 glycosylation on TYRP1 may also impact TYRP1 activity and/or stability.

Interestingly, the classical glycosylation pathway can be bypassed (Branza-Nichita et al., 2000). In mice, this process occurs when the endomannosidase pathway directly in the Golgi compartment is used, and this leads to a different glycan pattern (Negroiu et al., 1999).

In addition, TYRP1 is phosphorylated on five sites in human proteins (S46, S137, S207, T222, S270), and on one site in murine proteins (S535) (PhosphoSitePlus website (phosphosite.org)). These sites were assigned by mass spectrometry. Notably, only the serine in position 46 is not conserved between the human and mouse proteins. Their biological consequences of these sites remain to be elucidated.

After its synthesis in the endoplasmic reticulum and passage through the Golgi apparatus, the TYRP1 protein is transported through the endosomal recycling pathway, reaching the melanosomes through tubular-vesicular endosomal domains (Jani, Purushothaman, Rani, Bergam, & Setty, 2015). The melanocyte's ultimate goal is to deliver melanin pigment to the keratinocytes, where it acts as an "umbrella" in the keratinocyte nucleus, preserving or limiting DNA alterations in response to UV irradiation. The path from the endoplasmic reticulum to the keratinocyte is a multistep process involving many proteins, and this "TYRP1 route" is not yet completely clear (Raposo & Marks, 2007).

In most animal models, TYRP1 is involved in the synthesis of eumelanin, a photoprotective pigment (Boissy et al., 1996; Zhao, Eling, Medrano, & Boissy, 1996). However, its exact role in humans is not yet well-defined because of differing experimental results. Mouse Tyrp1 catalyzes the oxidation of 5,6-dihydroxyindole-2-carboxylic acid (DHICA), a major eumelanogenic intermediate (Jiménez-Cervantes et al., 1994; Kobayashi et al., 1994). *A contrario*, human TYRP1 does not express any DHICA oxidase activity (Boissy, Sakai, Zhao, Kobayashi, & Hearing, 1998). Winder et al. showed that mouse Tyrp1 could be a dopachrome tautomerase that catalyzes the conversion of L-dopachrome in DHICA, probably in cooperation with Dct/Tyrp2 (Winder, Wittbjer, Odh, Rosengren, & Rorsman, 1994). Another study reported that human and mouse TYRP1 have catalase activity that could play a role in decomposing hydroperoxide to avoid disrupting melanin and its precursors (Halaban & Moellmann, 1990). Other potential enzymatic functions in pigmentation have been ascribed to human and/or murine TYRP1, such as the hydroxylation of tyrosine and the oxidation of DOPA or 5,6-dihydroxyindole (DHI) (Sarangarajan & Boissy, 2001; Sarangarajan et al., 2000; Zhao, Zhao, Nordlund, & Boissy, 1994). Because the TYRP1 structure was recently solved (Lai et al., 2017), these different TYRP1 activities should be revisited. The authors provided evidence for the presence of two zinc ions in the active site, which makes it unlikely that human TYRP1 could be a redox enzyme.

TYRP1 has been shown to play a non-enzymatic role in pigmentation by affecting the stability of TYR, the main pigmentation enzyme. Murine Tyrp1 forms a dimer with Tyr and thereby stabilizes it (Kobayashi & Hearing, 2007; Kobayashi, Imokawa, Bennett, & Hearing, 1999). Human TYRP1 also forms a dimer with human TYR (Dolinska, Wingfield, Young, & Sergeev, 2019; Wu & Park, 2003). Tyrp1's enhancement of Tyr stability is lost with the Tyrp1 *brown* mutation form (guanine to adenine at position 503) (Manga et al., 2000). A previous report convincingly demonstrated that ectopic expression of human TYRP1 cDNA enhances TYR activity (Zhao et al., 1996). TYRP1 also contributes to melanosomal structure and maturation (Sarangarajan & Boissy, 2001). Mutation of *Tyrp1* that leads

to the mouse ‘*light*’ phenotype (hairs pigmented only at their tips) has been associated with disrupted melanosomal structures (Johnson & Jackson, 1992). Moreover, expression of C110Y (a mutated *brown* TYRP1) by cDNA overexpression causes abnormal melanosome structures (Sarangarajan & Boissy, 2001; Sarangarajan et al., 2000).

TYRP1 mutations can lead to the OCA3 phenotype, a form of albinism characterized by rufous or brown skin coloring as well as visual anomalies. Several *TYRP1* mutations have been identified and linked to this disease, which mainly occurs in the African population (Mártinez-García & Montoliu, 2013; Sarangarajan & Boissy, 2001; Simeonov et al., 2013) although OCA3-like cases have also been identified in Caucasian populations (Rooryck, Roudaut, Robine, Müsebeck, & Arveiler, 2006). In “brown” mutant mouse melanocytes (melan-b), Tyrp1 is not correctly processed, so the endoplasmic reticulum (ER) is retained (Toyofuku et al., 2001). Indeed, Toyofuku and colleagues hypothesized that OCA3 could be a Tyrp1 ER retention disease. In the Solomon Islands, over-representation of blond-haired people has also been associated with *TYRP1* mutation (Kenny et al., 2012). Abnormal expression and cellular localizations of TYRP1 proteins have also been described in Hermansky-Pudlak syndrome, a multisystemic disease that includes oculocutaneous albinism (Boissy et al., 2005; Helip-Wooley et al., 2007; Richmond et al., 2005).

In conclusion, TYRP1 is clearly involved in the pigmentation cascade, although the exact role of human TYRP1 proteins remains a matter of debate. Further investigations are also needed to better understand the role of TYRP1 in other cellular processes.

▪ **Noncoding functions of *TYRP1* mRNAs**

There are few studies concentrating on TYRP1 or DCT rather than the more popular TYR. In light of this, we recently demonstrated a noncoding function of *TYRP1* mRNA: promotion of melanoma growth (Gilot et al., 2017). This result is consistent with our previous publications, which show that high levels of *TYRP1* mRNAs in patients with metastatic melanomas are associated with poor clinical outcomes (El Hajj, Gilot, Migault, Theunis, van Kempen, et al., 2015; El Hajj et al., 2013; Ghanem & Journe, 2011; Journe et al., 2011). The “oncogenic role” of *TYRP1* mRNA has long been ignored, as authors almost exclusively associated *TYRP1* mRNA with the pigmentation process. However, two publications have suggested that *TYRP1* mRNA could be involved in processes other than pigmentation (Fang, Tsuji, & Setaluri, 2002; Vijayasaradhi, Doskoch, Wolchok, & Houghton, 1995). Indeed, Tyrp1 was implicated in melanocyte proliferation and survival (C. Y. Li et al., 2004). In addition, an RNAi screen showed that the *TYRP1* defect phenotype is characterized by G2 arrest (Kittler et al., 2007), although the TYRP1 protein or mRNA responsible for this phenotype has not yet been identified. In the same way, transfection of antisense oligonucleotides targeting *TYRP1* in melanocytes and melanoma cell lines

leads to G1 arrest, with increased apoptosis *in vitro* and decreased tumor growth *in vivo* (C. Y. Li et al., 2004). Collectively, these data suggest that the expression levels of *TYRP1* mRNA and/or TYRP1 proteins are associated with melanoma cell and/or melanocyte proliferation and survival.

In 2017, we showed that *TYRP1* mRNA sequesters a tumor suppressor miRNA, miR-16 (Gilot et al., 2017).

We began by demonstrating both *in vitro* and *in vivo* that *TYRP1* mRNA expression levels are tightly linked to melanoma cell proliferation and tumor growth. Interestingly, this effect is independent of TYRP1 proteins, in accordance with the results from the Institut Jules Bordet cohort (Journe et al., 2011).

We identified miR-16 and miR-155 as miRNAs associated with the 3'-UTR of *TYRP1* (Figure 5a). Importantly, miR-16 binds *TYRP1* through non-canonical MREs, with imperfect base-pairing between miRNA seed sequences (nucleotides 2-7) and MREs. The three identified non-canonical MRE-16s fail to induce *TYRP1* mRNA decay. Thus, miR-16 binding to *TYRP1* corresponds to a “long-term” sequestration, in contrast with the “short-term” mRNA binding (associated with mRNA decay). *TYRP1* mRNA is thus considered to be an endogenous miRNA sponge (Gilot & Galibert, 2018; Migault, Donnou-Fournet, Galibert, & Gilot, 2017).

We showed that the sequestration of miR-16 by *TYRP1* mRNA leads to the derepression of several miR-16 targets, including the *RAB17* mRNAs involved in cell proliferation and probably also tumor growth. In other words, by sequestering miR-16, *TYRP1* mRNA inhibits the miR-16 tumor suppressor function. By quantifying the expression levels of *TYRP1* mRNA and *RAB17* (an miR-16 target in cutaneous melanomas), it is possible to estimate the miR-16 sponge activity of *TYRP1*. Moreover, the combination of these two markers efficiently predicts the overall survival of metastatic melanoma patients in two independent cohorts. It is important to note that the expression levels of miR-16 are stable in these tumors, confirming that miRNAs can be expressed but inactive.

We demonstrated that sequestered miR-16 can be released from *TYRP1* mRNA. *In vivo*, released miR-16 exerts tumor suppressor activity by inducing the decay of the mRNA targets, including *RAB17*. These results suggest that “sequestered” miR-16 is not modified during this long-term binding, and that its targets remain reachable.

Target site blockers (TSB, antisense oligonucleotides) efficiently release miR-16 from *TYRP1* mRNA by masking the miR-16 binding sites on *TYRP1* mRNAs. TSBs are 16 to 20-nucleotides long (Hartmann et al., 2016; Messina et al., 2016; Wynendaele et al., 2010). To increase their target affinity and selectivity, these were synthesized as fully phosphorothioated DNA/LNA mix-mers. The distribution of locked nucleic acids ensures that the mix-mers do not catalyze RNase H-dependent degradation of the targeted RNAs. We designed a TSB (‘TSB-T3’) that selectively binds to the sequence overlapping one of the MRE-16s in the 3'-UTR of *TYRP1*. TSB-T3 masks MRE-16 and prevents the sequestration of miR-16 on *TYRP1* mRNA. We therefore showed that TSB-T3 decreases *TYRP1* miR-

16 sponge activity, and in turn, the freed miR-16 reduces the cell density of melanoma cell lines and tumor growth in a patient-derived xenograft model.

Altogether, our work suggests that *TYRP1* mRNAs could be an interesting target for melanoma therapy. Importantly, the efficiency of TSB-T3 in reducing cell density was correlated with *TYRP1* expression levels, and independent of the *BRAF*, *NRAS*, or *TP53* mutation states. This suggests that the selection criterion for applying such a treatment would be *TYRP1* levels, not the tumor's mutation status.

DISCUSSION

In the melanoma and pigmentation fields, TYRP1 is considered an “ugly duckling,” since mutations in the *TYRP1* gene have been associated just with the rare and untreatable OCA3 disease, and because the gene's role in the human pigmentation process is unclear (Sarangarajan & Boissy, 2001). Despite this, some researchers did not wait for the discovery of the noncoding functionality of *TYRP1* to consider it a “putative therapeutic target,” and different teams have tried to immunize melanoma patients using therapeutic antibodies that target TYRP1 (Hara, Takechi, & Houghton, 1995; Khalil et al., 2016; Patel et al., 2007). The 20D7 antibody (IMC-20D7S) reached a Phase I trial (Khalil et al., 2016), and this antibody was shown to reduce the tumor sizes of human melanoma cells xenografted onto nude mice. Despite the encouraging results, the use of this antibody is restricted to patients expressing TYRP1 proteins in the plasma membrane (Takechi, Hara, Naftzger, Xu, & Houghton, 1996). In our study, three different antibodies targeting various epitopes on TYRP1 were used, but importantly about half of the metastatic melanoma patients in the Institut Jules Bordet cohort had no detectable TYRP1 proteins at all (El Hajj et al., 2013). Nonetheless, the immunization strategy is of interest for melanoma using other targets/epitopes (Sahin et al., 2017, 2020). In contrast with TYRP1 proteins, *TYRP1* mRNA is detectable in almost all melanomas (Figure 2).

Gene expression patterns determine cell fate and cellular processes, including melanogenesis. MicroRNAs, by inducing RNA decay, sculpt the expression of most mRNAs. The regulation of miRNA activity is complex and so far has not been elucidated. The loss of alleles encoding miR-16 decreases miRNA counts per cell, which in turn disrupts gene expression. Abnormal miR-16 expression levels due to the deletion of the *DLEU2* locus are associated with prostate cancers, pituitary cancers, and chronic lymphocytic leukemia (Aqeilan, et al., 2010; Bottoni et al., 2005; Calin et al., 2008), which supports the importance of miR-16 expression and activity in a clinical context. On the other hand, miRNA expression is not always correlated with activity (Bartel, 2009). For instance, miRNA activity can be regulated by direct miRNA modifications such as m6A, which is not usually assessed in RNA-sequencing experiments (Bartel, 2018b; Konno et al., 2019). Indirect regulations can also modulate miRNA activity, and miRNAs have been shown to interact with different RNA classes including mRNAs, long noncoding RNAs, and circular RNAs (circRNAs).

The concept of competition between RNAs to bind to a limited amount of miRNAs was initially described in *Arabidopsis thaliana* (Franco-Zorrilla et al., 2007). This competing endogenous RNA model (Salmena, Poliseno, Tay, Kats, & Pandolfi, 2011) was extended to eukaryotes, but the concept is based on miRNA/RNA canonical base pairing. This implies that miRNA binding to an RNA target is transient, since the miRNA induces the decay of the RNA. Thus, two linear RNAs carrying the same MRE are competitors to bind the same pool of miRNAs, and convincing demonstrations of this have been obtained *in vivo* for the *PTEN* and *BRAF* pseudogenes (Karreth et al., 2015, 2011; Tay, Rinn, & Pandolfi, 2014). Put differently, the expression levels of two linear RNAs are closely correlated if we assume that RNA half-life is “exclusively” dependent on miRNA activity. When a linear RNA and a circRNA (carrying the same MRE) compete for miRNA binding, their expression levels are not strictly correlated, since circRNAs are not destroyed by the miRNA (due to its shape limiting the recruitment of RNase). Since the interaction between the circRNAs and miRNAs is prolonged, it is tempting to separate out these two forms. Thus, a circRNA might be considered to be an “miRNA sponge” (long interaction), while the linear RNA would be a “competitor RNA” (transient interaction).

In 2017, we added a level of complexity to the ceRNA model, investigating the role of non-canonical MREs. The non-canonical sites do not have six contiguous Watson-Crick pairs in the seed region, and instead have a wobble, mismatch, or single-nucleotide bulge. Increasing evidence indicates that miRNAs bind to a subset of RNAs without inducing their decay. In this case, the base pairing between the miRNA seed region and RNA is imperfect, as identified in the case of miR-16 and *TYRPI*. The biological role of these imperfect interactions is still matter of debate. It has been proposed that these interactions are artefactual, rare, and irrelevant since the miRNAs do not exert decay activity (Bartel, 2018a). Nevertheless, experiments based on cross-linking immunoprecipitation (CLIP) and other methods have been done by different teams, and they have all identified such interactions (Grosswendt et al., 2014; Hafner et al., 2010; Helwak, Kudla, Dudnakova, & Tollervy, 2013; Loeb et al., 2012; Luna et al., 2015; Seok, Ham, Jang, & Chi, 2016).

The main criticism of non-canonical base-pairing relies on the inability of the miRNA to induce RNA decay, suggesting that this type of interaction is not physiological. Moreover, it is well-accepted that miRNA binding to its binding sites is determined by the number of complementary bases between the miRNA and the targeted RNA. An miRNA binds preferentially to an 8mer-seed over a 7- or 6mer-seed (Bartel, 2018a). Surprisingly, pairing to the miRNA 3' region – in particular the nucleotides 13-16 – can supplement the imperfect base-pairing between miRNA seed sequences and RNA (due to a bulge) (Bartel, 2009), although this 3'-supplementary pairing seems to be rare (Grimson et al., 2007; Wee, Flores-Jasso, Salomon, & Zamore, 2012). Such compensatory base-pairing increases the affinity of the miRNA for its targets, and can be predicted by the RNAhybrid Webtool (Rehmsmeier, Steffen, Hochsmann, & Giegerich, 2004).

In this context, we identified three non-canonical base-pairing examples between miR-16 and the *TYRPI* 3'-UTR. We showed that miR-16 interacts with *TYRPI* but does not induce RNA decay.

Surprisingly, we demonstrated that miR-16 binding on these non-canonical MREs promoted *TYRPI* expression (RNA & protein). The microRNA acts as a rheostat, tuning *TYRPI* expression via non-canonical base-pairing. It is tempting to postulate that miR-16 promotes the translation of *TYRPI* mRNA through fragile X mental retardation syndrome-related protein 1 (FXR1). Vasudevan's team demonstrated that FXR1 promotes the circular shape of linear mRNAs, increasing their translation efficiency in non-canonical translation, and limiting the decay of their RNAs (Vasudevan, 2012; Vasudevan, Tong, & Steitz, 2007). This hypothesis is currently being investigated for *TYRPI* mRNA. We initially explored a particular localization of the miR-16/*TYRPI* complex promoting its translation as described for several RNAs located in P-bodies or stress granules. Despite thorough investigation, we have not been able to identify a characteristic *TYRPI* localization (unpublished data).

The ceRNA model is still being debated, as the stoichiometry of miRNA and target RNAs has been barely if at all investigated (Thomson & Dinger, 2016). Based on absolute quantifications of miR-16, *TYRPI*, and *RAB17* calculated for understanding the miRNA regulation of *TYRPI*, we plan to upgrade the ceRNA model with the non-canonical MRE. Moreover, in the literature the difference between competition and sequestration is not clear. We previously summarized the criteria for miRNA sponges (Migault et al., 2017). Briefly, a sponge should be highly expressed to sequester all targeted miRNAs, and it ideally should contain several imperfect miRNA binding sites per RNA molecule, as with linear *TYRPI* mRNA. The sequestration of miRNA has also been illustrated *in vivo* in circRNAs (Hanniford et al., 2020; Hansen, Kjems, & Damgaard, 2013; Kleaveland, Shi, Stefano, & Bartel, 2018; Kristensen et al., 2020; Memczak, Jens, Elefsinioti, & Torti, 2013), confirming the physiological relevance of this type of miRNA regulation. Due to their circular shape, canonical miRNA binding sites are compatible with the miRNA-sponging activity of these circRNAs. However, well-characterized miRNA sponges are rare in the literature because of the highly specific circRNA expression patterns and the difficulties inherent in predicting imperfect base-pairing between miRNA and linear RNA (sponges) using current algorithms. We investigated the expression levels of circRNAs derived from the *TYRPI* gene, but we did not find this circRNA in melanoma cell lines (unpublished data). Nevertheless, we were able to decipher how *TYRPI* mRNA exerts its indirect oncogenic role in melanoma, and we validated an antisense oligonucleotide approach to restoring miR-16 activity. However, the actors responsible for miR-16 sequestration of *TYRPI* remain unknown. The process might involve proteins such as an RBP or another noncoding RNA, but further investigations are needed to elucidate this "druggable" mechanism.

MicroRNA sponges (circRNAs) were recently reviewed in melanoma (Hallajzadeh et al., 2020). However, how many miRNA sponges exist? Their identification remains laborious, and we have previously reviewed the approaches to identifying such RNAs (Migault et al., 2017). The sponge concept could be extended to the sponging of RBPs such as ELAVL1 (HuR) (Kim et al., 2016) and transcription factors (Sigova et al., 2015). In this way, the identification of miRNA and protein sponges

might lead to an improved understanding of cancer biology, as few “driver mutations” have been found for cancers such as triple wild-type cutaneous melanoma.

Elucidating driver mutations is crucial for elaborating therapeutic strategies, but the identification of tumor suppressor activity losses is also needed. In melanomas, miR-16 is highly expressed, but its activity is impaired due to its sequestration of *TYRP1*. As miR-155 can induce *TYRP1* RNA decay, this microRNA is considered a normal brake in miR-16 sequestration. In this way, miR-155 reduces the expression levels of both *TYRP1* mRNAs and proteins, consequently restoring miR-16 activity. Collectively, these data support a key role for miR-155 in the pigmentation process, and suggest that miR-155 acts as a rheostat to optimize *TYRP1* expression in a local adaptation to differing UV radiation levels along the earth’s latitudes (J. Li & Zhang, 2013). The discovery of MRE-155 on *MITF* further supports miR-155’s rheostatic role in pigmentation (Arts et al., 2015; Zhang et al., 2012). miR-155 promotes the degradation of the master gene involved in pigmentation and reduces the expression levels of *TYRP1* mRNA. The question remains as to why miR-155 targets *MITF* and *TYRP1*, since *MITF* decay should be sufficient to downregulate the pigmentation cascade. Notably, the effect of miR-155 on *MITF* has been demonstrated using synthetic miR-155. In those experiments, intracellular miR-155 reached a non-physiological level, and we estimated that miR-155 is poorly expressed in melanoma cells (<100 copies/cell). Additional investigations are therefore required to elucidate the exact role of miR-155 in the pigmentation process

Due to its role in several cancers, miR-155 is considered by the miRNA community as an oncomir, an miRNA involved in oncogenesis (Di Leva, Garofalo, & Croce, 2014; Volinia et al., 2006). However, in melanoma, miR-155 likely acts as a tumor suppressor (Levati et al., 2009; Segura et al., 2010). The overexpression of synthetic miR-155 reduces the proliferation and viability of melanoma cells in culture (Gilot et al., 2017). But due to the oncogenic activity of miR-155 in many cell types, patients with metastatic melanoma cannot be treated using a synthetic version of miR-155, as previously attempted with miR-34 (Beg et al., 2017; Rupaimoole & Slack, 2017). Similarly, the injection of miR-16 should impede the cell proliferation of every cell in the body, a strategy comparable to chemotherapy, and thus does not present any interest *in vivo*. In contrast with these systemic injections of tumor suppressing miRNAs, target site blockers are specific to the cells expressing the RNA (here melanoma cells and *TYRP1*). Moreover, TSBs do not modify miRNA expression levels, favoring miRNA availability and thus activity. In this way, TSBs restore normal miRNA activity in cancer cells, reducing cell proliferation and survival. Ultimately, the extensive identification of miRNA sponges should offer new therapeutic targets, because TSB and ASO generally arise in clinic (Quemener et al., 2020). Several ASOs have recently been FDA-approved (Spinraza, Eteplirsén, etc.), demonstrating the efficacy and safety of these in humans (Crooke, Witztum, Bennett, & Baker, 2018).

The MITF protein has emerged as a key coordinator of many aspects of the pigmentation process and melanoma biology (Goding & Arnheiter, 2019). Because it governs pigmentation gene expression,

MITF is considered to be an anti-melanoma transcription factor. MITF protein levels and activity are decreased by the dysregulation of signaling pathways in cutaneous melanomas, a result of genetic alterations such as the BRAF-V600E mutation. Consequently, melanoma cells produce less eumelanin, and protection against UV radiation decreases (Hemesath et al., 1994). The drop in MITF activity is associated with a dedifferentiation process and increased invasion capacity (Hoek et al., 2008, 2006; Hoek & Goding, 2010b). MITF protein levels differ greatly among the melanoma cells inside tumors. Intriguingly, cells with low MITF levels proliferate slowly, but constitute an invasive subpopulation of tumor cells (Hoek & Goding, 2010a). This concept of phenotype-switching was originally published by Hoek et al. (Hoek et al., 2008, 2006) and was recently updated (Rambow, Marine, & Goding, 2019). Authors proposed that the development of an effective anti-melanoma strategy therefore requires the monitoring and management of subpopulations, including differentiation state gradients. Recent single-cell RNA-sequencing (scRNA-seq) experiments confirmed that relapse is driven by a small subpopulation of residual or drug-tolerant cells (Rambow et al., 2018) corresponding to the minimal residual disease (MRD). scRNA-seq results have highlighted multiple distinct and coexisting drug-tolerant transcriptional states that are associated with MRD, including cells with low or high MITF expression. Recently, two reviews summed up these experiments, concluding that high MITF-expressing cells (MITF^{HI}) participate in relapse (Bai et al., 2019; Rambow et al., 2019). The authors proposed that these hyper-differentiated cells are drug-tolerant, and that they can be induced by targeted therapy as described for other cell populations (starved and undifferentiated states). The degree of interdependency between therapy-resistant subpopulations is currently not known. The MITF^{HI} state may confer drug tolerance to neighboring cells in the manner of an ECE1-EDN1 (endothelin-converting enzyme 1–endothelin 1) paracrine (Smith et al., 2017). Targeting these MITF^{HI} cells may thus prevent the emergence of a specific “undifferentiated” state. In accordance with this idea, Rambow et al. proposed that ablation of the “starved” melanoma cell subpopulation may be sufficient to provoke the collapse of the entire MRD lineage tree (Rambow et al., 2018).

MITF has long been considered as an oncogene because it is sometimes overexpressed in human melanomas. Recently, we added another reason for this classification (Gilot et al., 2017). We showed that high expression levels of *TYRP1* mRNA are associated with poor survival due to the miR-16 sponge activity of *TYRP1*. Indirectly, by regulating *TYRP1*, MITF induces the loss of miR-16 tumor suppressor activity. Altogether, these publications define the role of the MITF transcription factor somewhere between the promotion of malignant behavior and the channeling of melanocytes towards terminal differentiation and/or pigmentation.

The relationship between pigmentation and melanoma remains unclear. *TYRP1* gene expression gives rise to two products. On one hand, it produces an mRNA whose sponge activity is correlated with poor melanoma patient survival and which is involved in melanoma progression. Conversely, it also produces a protein that is involved in pigmentation and which protects against the onset of melanoma.

This finding is rather paradoxical, and further investigations are needed to better understand these observations.

Revised manuscript

FIGURE LEGENDS

Figure 1: Human *TYRP1* gene and its products.

Human *TYRP1* gene, transcript (a) and protein (a, b) details according to Ensembl.

Figure 2: *TYRP1* mRNA expression.

(a) RNAseq analysis of human *TYRP1* expression in different tissues from the Human Protein Atlas dataset. Results are expressed in transcripts per million kilobases). (b) *TYRP1* mRNA expression in 10,967 tumor samples from the TCGA Pan-Cancer Atlas (www.cbioportal.org). (c) *TYRP1* expression in TCGA melanoma biopsies ($n = 459$ melanoma samples). To illustrate the expression heterogeneity of pigmentation-related mRNAs, the genes *TYR*, *DCT*, *MITF*, and *MLANA* were also analyzed. These results are based upon data generated by the TCGA research network (<http://cancergenome.nih.gov/>).

Figure 3: Schematic representation of the human *TYRP1* promoter and enhancer with *cis*-acting elements.

(a) Transcription factors bind the *TYRP1* enhancer and promoter at specific binding sites. Some have been studied in mouse or human models or in both. Notably, the M-box is green. Tbx2 and Pax3 binding sites in the human *TYRP1* promoter were predicted according to their binding sites from a murine model, and these predicted sites are in orange italics. OTX2 binding sites are depicted for retinal pigment epithelium cells. (b) A schema showing the role of MITF, SOX10, and BRG1 in the *TYRP1* promoter. In differentiated cells, this promoter becomes accessible to MITF. SOX10 binds to the enhancer and recruits BRG1 to form a chromatin loop. Consequently, these three proteins are able to transactivate the *TYRP1* promoter (Marathe et al., 2017).

Figure 4: 3'-UTR variability of *TYRP1* mRNA.

(a) Three mRNA variants have been successively used as reference sequences in the NCBI RefSeq database, with the NM_000550.3 isoform being used currently. This variant is distinguished from NM_000550.1 by the existence of four SNPs and two short deletions. (b) Distribution of the four SNPs in the CEU cohorts CEPH (Utah Residents with Northern and Western European Ancestry), JPT

(Japanese in Tokyo, Japan), and YRI (Yoruba in Ibadan, Nigeria), as per <https://www.ncbi.nlm.nih.gov/projects/SNP> and ensembl.org/Homo_sapiens/Variation/Population.

Figure 5: Impact of SNPs on *TYRP1* mRNA decay.

(a) Relative positions of microRNA response elements (MREs) and the SNPs rs683 and rs910 on the *TYRP1* 3'-UTR. (b) These SNPs modify the base-pairing between miR-155 and *TYRP1* alleles. (c) Table showing the consequences of these SNPs on *TYRP1* decay and expression as a function of *TYRP1* allele and patient origin.

Figure 6: *TYRP1* protein domains and post-translational modifications.

TYRP1 contains a signal-peptide region, cystein-rich regions, two metal-binding domains, and a unique transmembrane region. Phosphorylation and glycosylation sites were obtained from www.phosphosite.org and www.uniprot.org.

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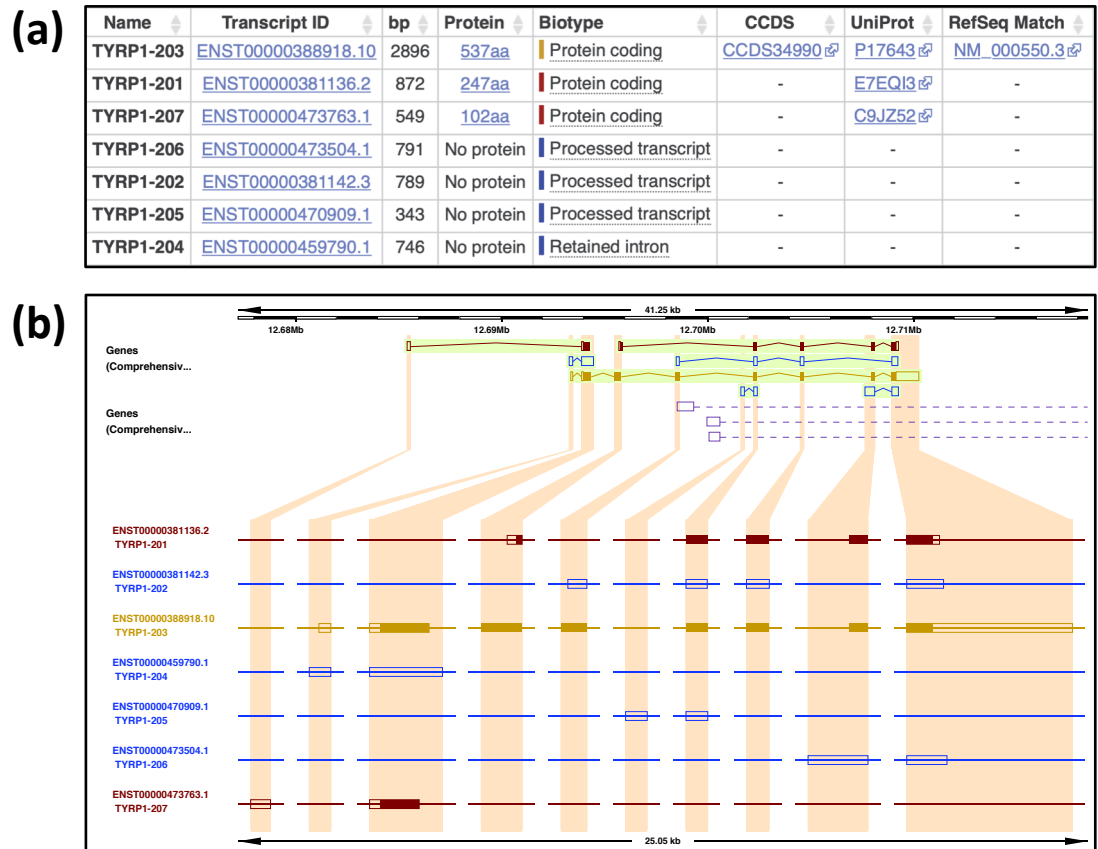


Figure 1

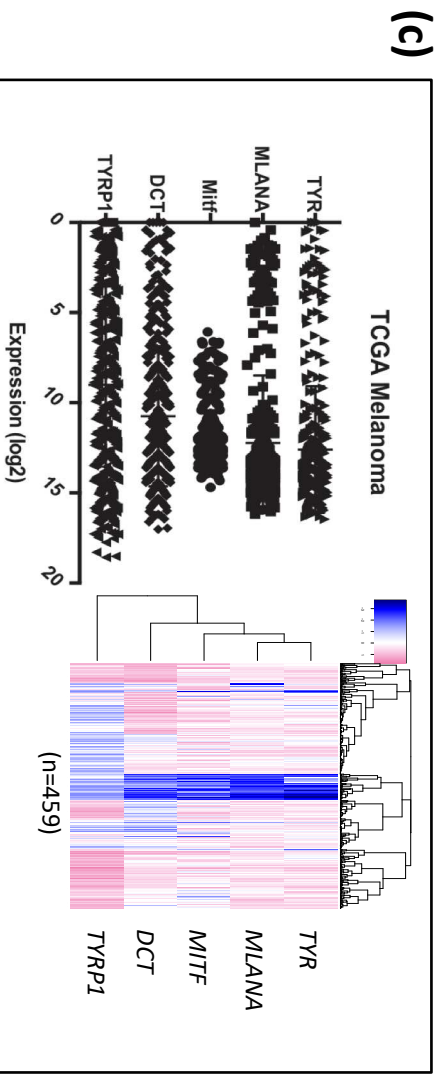
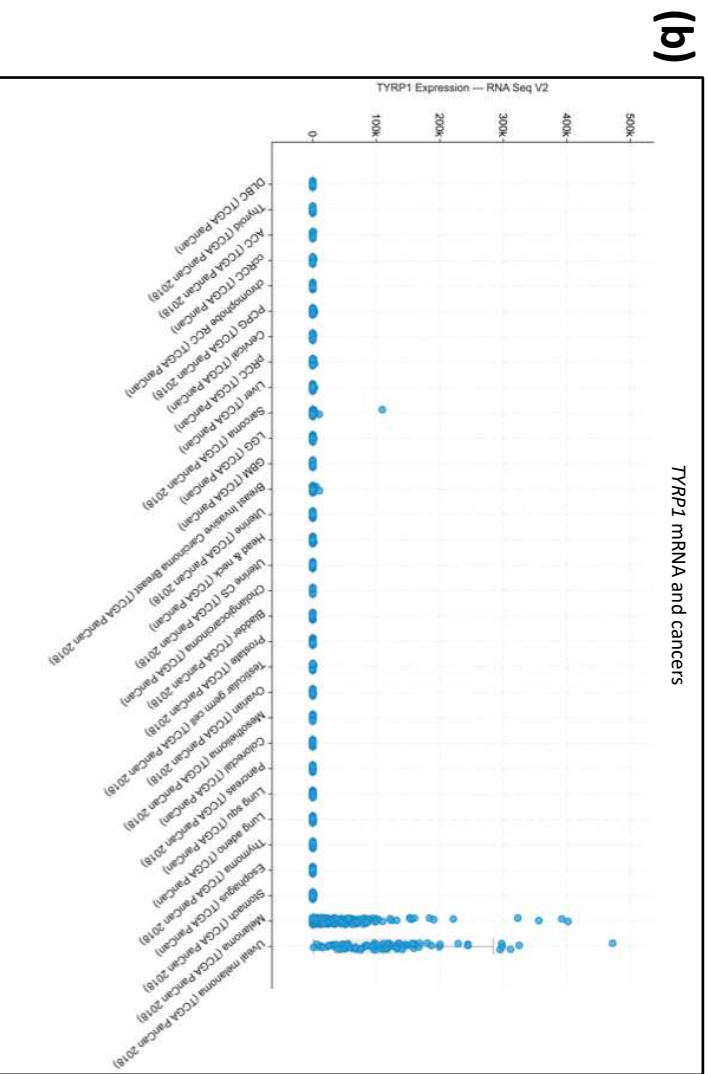
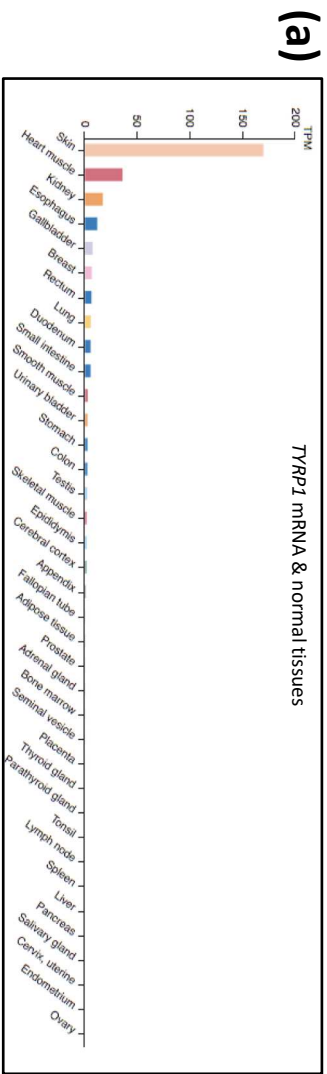
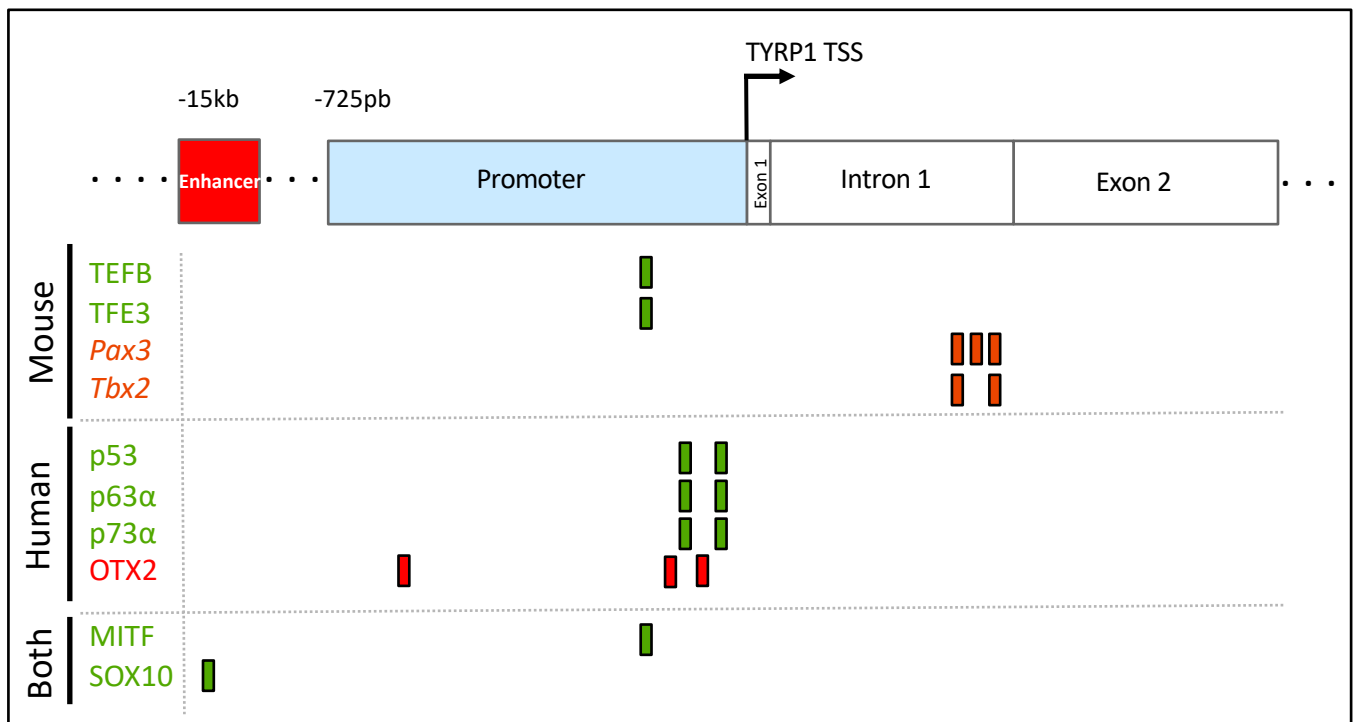


Figure 2

(a)



(b)

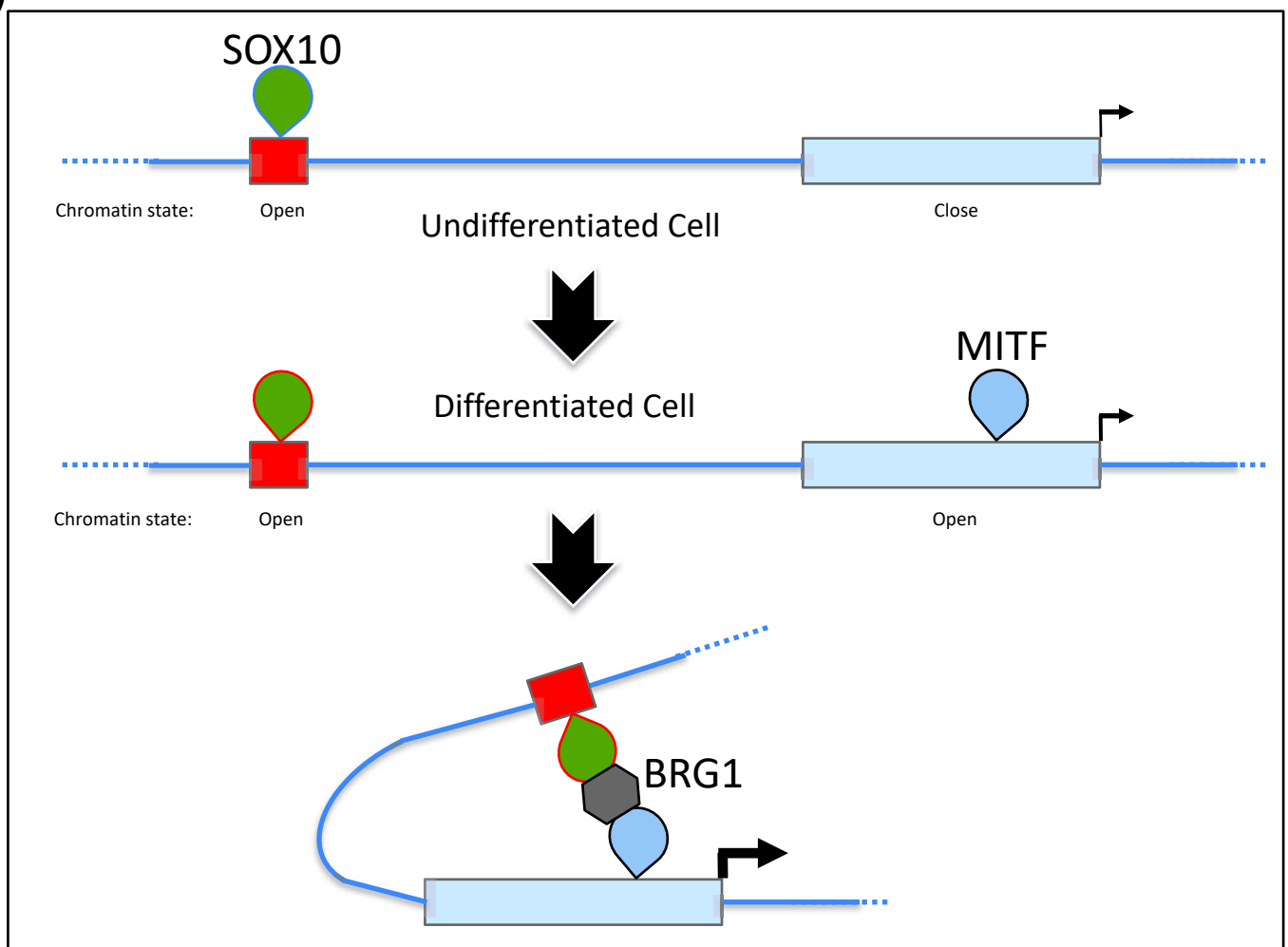


Figure 3

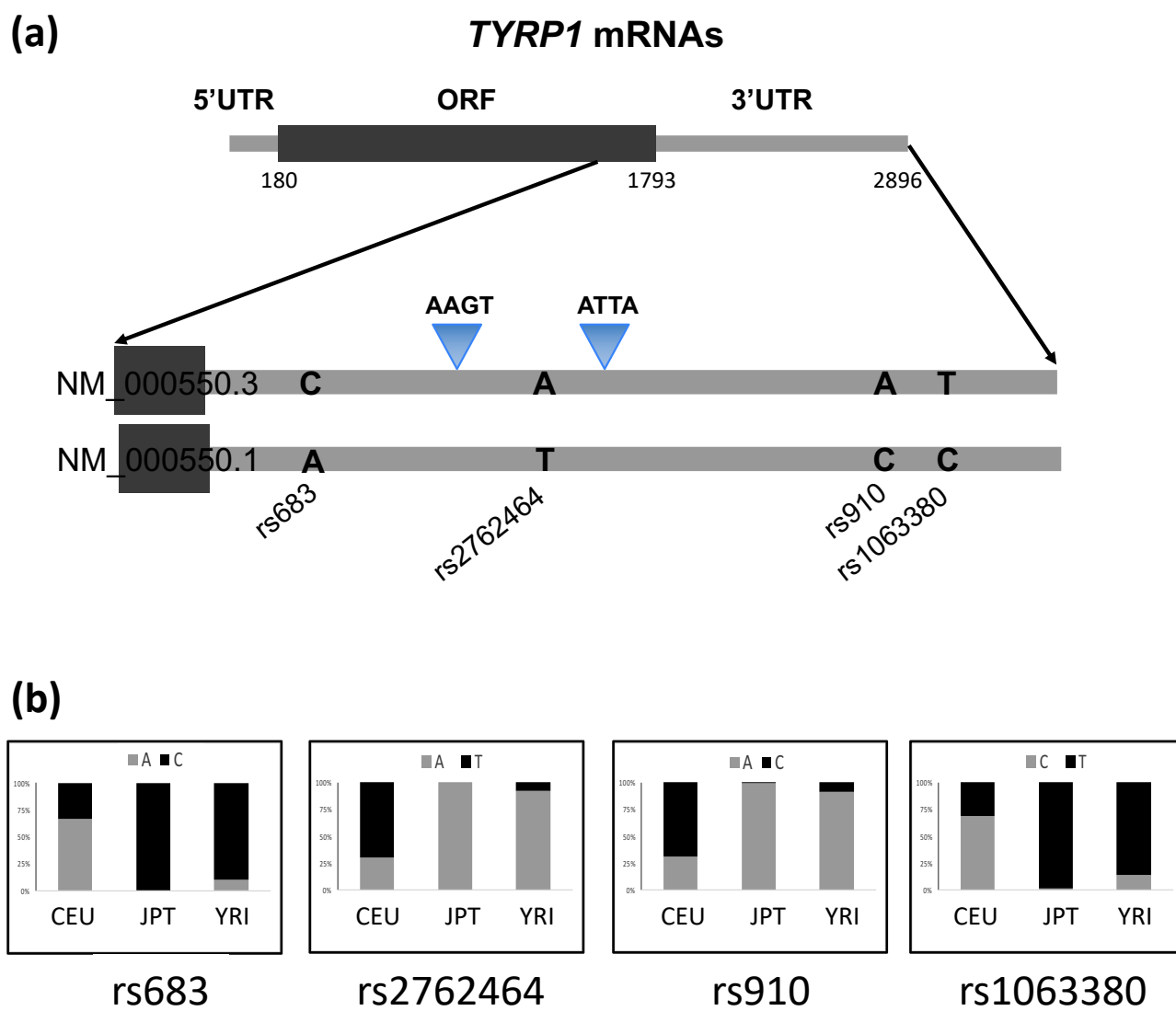
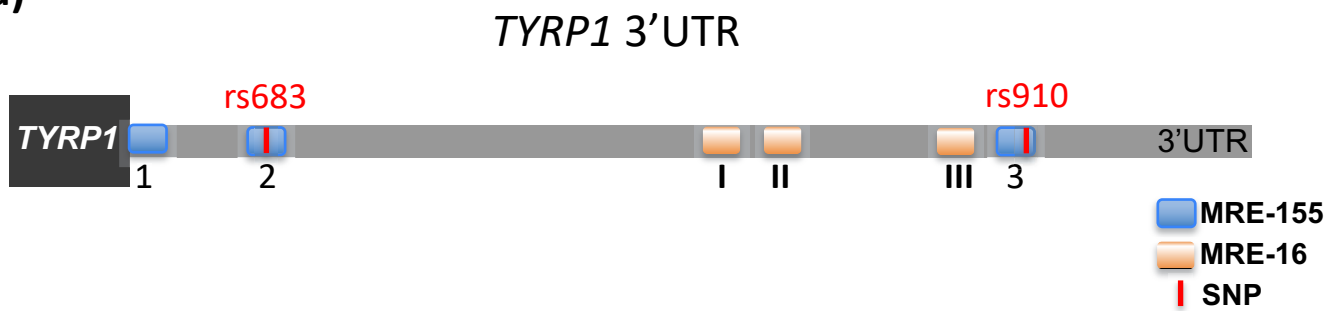


Figure 4

(a)



(b)

	rs683	rs910
NM_000550.3 miR-155-5p NM_000550.1	<pre> UUAGCAUUA ••••• UG...GCUAAUCGUAUU ••••• UUAGAAUUA</pre>	<pre> UAGCAUUUA ••••• UG.....GCUAAUCGUAUU ••••• UAGCAUUC</pre>

(c)

	JPT & YRI	CEU
cutaneous melanoma risk	Low	High
<i>TYRP1</i> allele	NM_000550.3	NM_000550.1
miR-155 mediated Decay	High	Low
<i>TYRP1</i> half-life	Low	High
miR-16 sequestration	Low	High

Figure 5

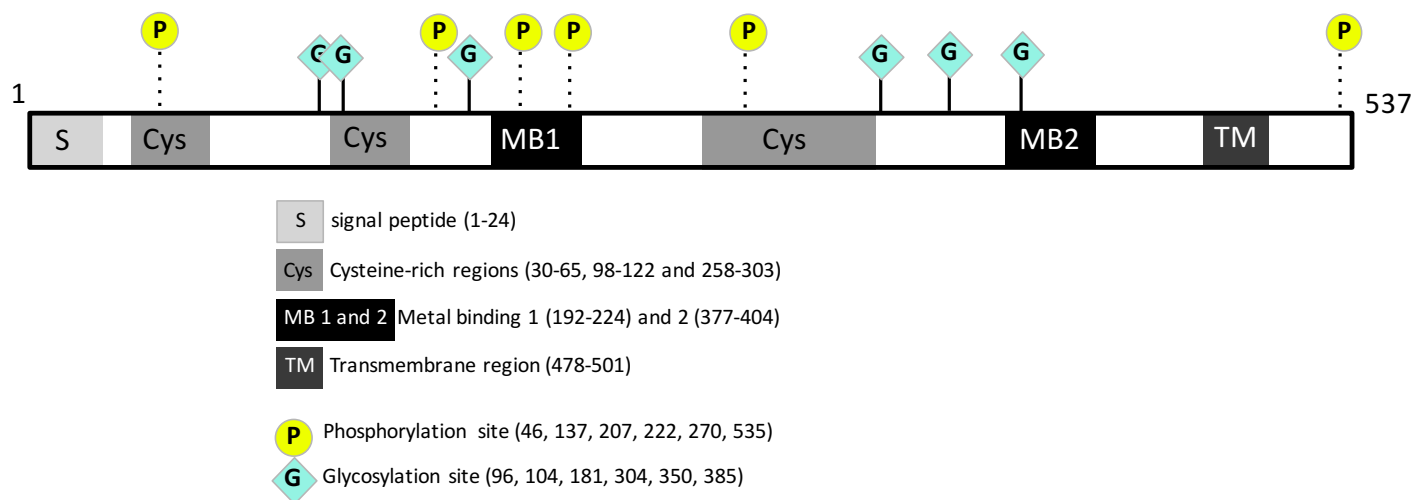


Figure 6

Table 1 : Direct activators and repressors of *TYRP1* expression

Factor	Role	Model		Reference
		Mouse	Human	
MITF	Activator	X	X	(Bertolotto et al., 1998; Shibata et al., 1992; Yasumoto et al., 1997)
TFEB	Activator	X		(Verastegui et al., 2000)
TFE3	Activator	X		(Verastegui et al., 2000)
Pax3	Activator	X		(Galibert et al., 1999)
Tbx2	Repressor	X		(Carreira et al., 1998)
p53	Activator		X	(Nylander et al., 2000)
p63 α	Activator		X	(Nylander et al., 2000)
p73 α	Activator		X	(Nylander et al., 2000)
Sox10	Activator	X	X	(Murisier et al., 2006)
OTX2	Activator		X	(Martínez-Morales et al., 2003)