Gyrfalcons *Falco rusticolus* adjust *CTNS* expression to food abundance: a possible contribution to cysteine homeostasis

Ismael Galván¹ · Ângela Inácio² · Ólafur K. Nielsen³

¹Department of Evolutionary Ecology, Doñana Biological Station - CSIC, Seville, Spain

²Laboratory of Genetics, Institute of Environmental Health (ISAMB), School of Medicine, University of Lisbon, Lisbon, Portugal

³Icelandic Institute of Natural History, Garðabær, Iceland

*Author for correspondence. Email: galvan@ebd.csic.es. Phone: +34954466700. Fax: +34954621125.

Author contribution statement IG conceived and designed this study, conducted laboratory work, analyzed the data and wrote the manuscript. ÂI designed gene primers and participated in laboratory work. ÓKN conducted fieldwork.

Abstract Melanins form the basis of animal pigmentation. When the sulphurated form of melanin, termed pheomelanin, is synthesized, the sulfhydryl group of cysteine is incorporated to the pigment structure. This may constrain physiological performance because it consumes the most important intracellular antioxidant (i.e. glutathione, GSH), of which cysteine is a constitutive amino acid. However, this may also help avoid excess cysteine, which is toxic. Pheomelanin synthesis is regulated by several genes, some of them exerting this regulation by controlling the transport of cysteine in melanocytes. We investigated the possibility that these genes are epigenetically labile regarding protein intake and thus contribute to cysteine homeostasis. We found in the Icelandic population of gyrfalcon *Falco rusticolus*, a species that pigments its plumage with pheomelanin, that the expression of a gene regulating the export of cystine out of melanosomes (*CTNS*) in feather melanocytes of developing nestlings increases with food abundance in the breeding territories where they were reared. The expression of other genes regulating pheomelanin synthesis by different mechanisms of influence on cysteine availability (*Slc7a11* and *Slc45a2*) or by other processes (*MC1R* and *AGRP*) were not affected by food abundance. As the gyrfalcon is a strict carnivore and variation in food abundance mainly reflects variation in protein intake, we suggest that epigenetic lability in *CTNS* has evolved in some species because of its potential benefits contributing to cysteine homeostasis. Potential applications of our results should now be investigated in the context of renal failure and other disorders associated with cystinosis caused by *CTNS* dysfunction.

Keywords *CTNS* · cysteine homeostasis · food abundance · gyrfalcon · pheomelanin

Introduction

Vertebrate pigmentation mainly depends on several genes that regulate the synthesis, structure and transport of melanins in melanocytes (Hubbard et al. 2010; Hearing 2011). During the synthesis of the sulphurated form of melanin (i.e. pheomelanin), which is responsible for orange-reddish animal coloration, the sulfhydryl group of the amino acid cysteine is incorporated to the pigment structure (García-Borrón and Olivares Sánchez 2011). As pheomelanin is then transferred to inert integumentary structures of the skin, feathers or hairs, cysteine used for pheomelanin synthesis is no longer available as an antioxidant as part of the tripeptide glutathione (GSH). As a consequence, pheomelanin synthesis represents a consumption of the most important intracellular antioxidant (Panzella et al. 2014). This consumption of cysteine/GSH contributes to some disorders, including melanoma and malignant glioma in humans (Panzella et al. 2014; Robert et al. 2015; Wang et al. 2016) and constraints to physiological performance in animals exposed to environmental sources of oxidative stress (Karell et al. 2011; Galván et al. 2011, 2014). However, cysteine is toxic if in excess, thus its consumption during pheomelanogenesis may also be advantageous under environmental conditions leading to excess cysteine, which may indeed explain the evolution of pheomelanin-based pigmentation (Galván et al. 2012).

The expression of genes regulating pheomelanin synthesis should therefore be key in determining the double-sided nature of pheomelanin-based pigmentation, in interaction with environmental factors. Some of us have recently found that zebra finches *Taeniopygia guttata* developing feathers pigmented by pheomelanin downregulate the expression of the gene *Slc7a11* (solute carrier family 7 member 11), which codes for the plasma membrane cystine/glutamate exchanger xCT that transports cystine (the cysteine dimer) to melanocytes to produce pheomelanin (Chintala et al. 2005), under exposure to a pro-oxidant substance (Galván et al. 2017). As this downregulation of *Slc7a11* avoided

cellular oxidative stress exerted by the pro-oxidant, it means that some species have evolved an epigenetic lability in *Slc7a11* that reduces cysteine consumption for pheomelanin production under stressful environmental conditions, when cysteine/GSH are most required to combat oxidative stress (Galván et al. 2017). Similar to this influence of environmental oxidative stress on gene expression, epigenetic permeability of genes regulating pheomelanin synthesis to environmental conditions that produce excess cysteine should also be physiologically advantageous. However, this possibility has never been explored under natural variations in dietary cysteine levels (i.e. food abundance) as a potential adaptive strategy.

Cysteine is a semi-essential amino acid, thus diet is an important source of cysteine for animals (Klasing 1998; Stipanuk et al. 2006). Excess cysteine can occur when its content in diet is higher than needed for protein synthesis (Stipanuk et al. 2006). Under such conditions, the toxicity of excess cysteine is produced by its autoxidation to cystine, which generates hydrogen peroxide, hence decreasing GSH levels (Viña et al. 1983) and causing oxidative DNA damage (Park and Imlay 2003). This causes several diseases in mammals and metabolic acidosis and disorders such as thinning of egg shells and poor growth in birds (Klasing 1998). However, cysteine is essential for protein structure, hence the importance of maintaining cysteine homeostasis. The enzyme cysteine dioxygenase (CDO) catalyzes the addition of molecular oxygen to the sulfhydryl group of cysteine to form less toxic products such as sulfate and taurine (Stipanuk et al. 2006). Nevertheless, the capacity of CDO to maintain cysteine homeostasis is probably limited, as derived from the fact that cystinosis, a disease characterized by the intralysosomal accumulation of cystine, is only explained by dysfunction in the cystine/H⁺ symporter (cystinosin) that exports cystine out of lysosomes (Chiaverini et al. 2012). Pheomelanin synthesis may also affect, together with CDO, cysteine homeostasis (Galván et al. 2012). In fact, cystinosin and pheomelanin are intrinsically associated, as the gene encoding cystinosin (*CTNS*) is

expressed in the organelles where melanin synthesis takes place in melanocytes (i.e. melanosomes) and thus inhibits pheomelanogenesis by promoting the efflux of cystine (Chiaverini et al. 2012).

Potential epigenetic mechanisms effective in avoiding excess cysteine may therefore be related to changes in the expression of *Slc7a11* and *CTNS*. Here we test these possibilities in a wild population of gyrfalcons *Falco rusticolus* exposed to different food regimes in Iceland, as these birds synthesize pheomelanin to pigment the feathers of their first plumage (Galván and Jorge 2015). As the gyrfalcon is a strict carnivore, variation in the food abundance to which nestlings are exposed during growth mainly reflects variation in protein availability in the diet (Klasing 1998). We also considered the gene *Slc45a2* (solute carrier family 45 member 2), which has also been suggested to affect pheomelanin synthesis by transporting cysteine into melanosomes (Xu et al. 2013). Lastly, we considered the melanocortin-1 receptor (*MC1R*) and agouti-related protein (*AGRP*) genes, which affect pheomelanin synthesis without influencing cysteine/GSH availability (Nadeau et al. 2008). We thus predicted that, if epigenetic sensitivity to protein availability has evolved in *Slc7a11* and *CTNS* (and possibly *Slc45a2*) as an adaptive mechanism to avoid excess cysteine, the expression of these genes should be adjusted to food abundance while *MC1R* and *AGRP* should not be affected.

Methods

Field methods

The study was conducted during two consecutive breeding seasons (June 2015 and 2016) in 16 gyrfalcon territories in Iceland. Nests were entered when the nestlings were 16-35 days old. Nestlings were ringed, weighed and the following morphometrics taken: flat chord of the wing, culmen and length of central tail feathers. Sexing was based on morphometrics. Ageing was based on length of central tail feather (Ó.K. Nielsen,

130 unpublished data). Body condition was calculated as the residuals of body mass
131 regressed against wing chord, culmen length and age. The study comprised a total of 47
132 gyrfalcon nestlings (Online Resource 1).

133 One or two growing body feathers were plucked from the back of nestling
134 gyrfalcons and immersed in RNAlater solution (Ambion, Thermo Fisher Scientific,
135 Waltham, MA) to stabilize and protect RNA, and stored at -20 °C until the analyses. These
136 feathers are dark brown with rusty-yellowish patches (Fig. 1). The yellowish coloration is
137 produced by pheomelanin, while the brown coloration is produced by the non-sulphurated
138 form of melanin (i.e. eumelanin; Galván and Jorge 2015).

139

140 **Estimation of food abundance**

141 Two or three collecting trips were made to each nest during the season, the last after the
142 young had fledged. During each visit all remains were collected, including all bones and
143 pellets, and also some feathers. For the analysis, remains were arranged according to
144 species, age groups and type, from which we estimated the number and biomass of prey
145 [see Nielsen (2003) for details]. Number and biomass of prey were thus considered as two
146 indices of food abundance to which nestling gyrfalcons were exposed during growth.

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148 **Gene expression in melanocytes**

149 During feather development, melanocytes at the dermal papillae show intense
150 melanogenesis (Lin et al. 2013). Therefore, the bottommost portion of the feather follicles
151 corresponding to the melanin unit, which represents an important reservoir of melanocytes
152 like in hair follicles (Mohanty et al. 2011), was isolated. RNA obtained from these samples
153 therefore correspond to melanocytes to a large extent. All follicular melanin units obtained
154 from each gyrfalcon nestling were pooled. Total RNA was extracted using TRI Reagent
155 (Ambion), and residual genomic DNA carry over was removed using the TURBO DNA-free

156 kit (Ambion). Complementary DNA (cDNA) was prepared from total RNA using RevertAid
 157 Reverse Transcriptase provided in the RevertAid First Strand cDNA Synthesis kit (Thermo
 158 Scientific, Thermo Fisher Scientific). qPCR was thus performed on cDNA for the genes
 159 *CTNS*, *Slc7a11*, *Slc45a2*, *MC1R* and *AGRP*. Reactions were performed using SYBR
 160 Green I Master in a LightCycler 480 System (Roche, Basel, Switzerland). The
 161 housekeeping glyceraldehyde-3-phosphate dehydrogenase (*GAPDH*) gene was used for
 162 normalization of cDNA samples. Cycle threshold (C_t) levels were used as a measure of
 163 gene expression, with lower C_t values indicating higher mRNA levels and higher gene
 164 expression levels. Two replicates were obtained for each sample, and the mean value was
 165 used in the statistical analyses. We did not obtain any amplification of genes from 8
 166 nestlings in the case of *CTNS*, from 6 nestlings in the case of *Slc7a11*, from 7 nestlings in
 167 the cases of *Slc45a2*, *MC1R* and *GAPDH*, and from 10 nestlings in the case of *AGRP*
 168 (Online Resource 1).

169 Gene primers were designed based on *Falco cherrug* refseq sequences (GenBank)
 170 using the Primer-BLAST tool (<https://www.ncbi.nlm.nih.gov/tools/primer-blast/>). The
 171 oligonucleotide primer sets used were as follows: 5'-GCTGGGGAATTGCTGCTTT-3' and
 172 5'-TGCAAC GAAGAACATCCTGG-3' for *Slc7a11*; 5'-CATGCCATCCCACTATCGCT-3'
 173 and 5'-GAGGCGCATAAGGACTACCC-3' for *Slc45a2*; 5'-GGGAGAAGCCGTGAGCTTT-3'
 174 and 5'-CAGTGAGTCCATCACCAGGC-3' for *CTNS*; 5'-CTCCTTCCTGGGGGTCATC-3'
 175 and 5'-TGATGAAGATGGTGCTGGAGA-3' for *MC1R*; 5'-GAAGCCAACGGTGACCTCAT-
 176 3' and 5'-AAGGCATTGAAGAAGCGGCA-3' for *AGRP*; and 5'-
 177 GGACCAGGTTGTTTCCTGTG-3' and 5'-TCCTTGGATGCCATGTGGA-3' for *GAPDH*.

178

179 **Statistical analyses**

180 We tested if gene expression levels (C_t) changed with food abundance using linear mixed-
 181 effects models (LMM) fit with restricted maximum likelihood (REML) estimation in R

environment (R Core Team 2017). As the two indices of food abundance (i.e. number and biomass of prey) were strongly correlated ($r = 0.98$, $N = 19$, $P < 0.0001$), we conducted separate models for these variables. LMM's thus included number or biomass of prey, in addition to sex and body condition, as fixed terms. Territory identity was added as a random factor to account for the common origin of gyrfalcon nestlings belonging to the same nests. Different models were fit for each gene. Effect sizes (b) were calculated with the library *lme4*, and P-values were calculated from the analysis of deviance of the models using the library *car*. All continuous variables were $\log_{10}$ -transformed prior to analyses to achieve normality assumptions.

Results

The effects of sex and body condition were not significant in explaining variation in the expression of any gene (sex: $0.09 < \chi^2_1 < 1.16$, $0.282 < P < 0.763$; condition: $0.00 < \chi^2_1 < 1.61$, $0.205 < P < 0.998$), and were thus removed from the models. There was a strong significant and negative effect of the number of prey on C_t for the gene *CTNS* ($b = -0.11$, $\chi^2_1 = 9.42$, $P = 0.002$), and a similar effect of the biomass of prey ($b = -0.11$, $\chi^2_1 = 8.38$, $P = 0.004$). This means that *CTNS* expression increased with the number of prey and with the biomass of prey available to gyrfalcon nestlings (Fig. 2). These significant effects did not change if sex and condition were kept in the models. In contrast, no index of food abundance was significant in the models for the other genes (*Slc7a11*: $0.48 < \chi^2_1 < 1.11$, $0.292 < P < 0.489$; *Slc45a2*: $0.39 < \chi^2_1 < 0.80$, $0.372 < P < 0.531$; *MC1R*: $0.01 < \chi^2_1 < 0.35$, $0.553 < P < 0.914$; *AGRP*: $2 \times 10^{-4} < \chi^2_1 < 0.01$; $0.942 < P < 0.990$).

Discussion

Although modulation of *CTNS* expression in response to intracellular cysteine levels has been shown *in vitro* in human kidney cells (Bellomo et al. 2010), our findings represent the

208 first evidence of epigenetic lability in the gene *CTNS* as response to a natural variation in a
209 dietary source of cysteine. Diet constitutes an important source of cysteine, and
210 consequently elicits the modulation of the enzyme CDO to maintain cysteine homeostasis
211 (Stipanuk et al. 2009). Protein intake therefore affects overall cysteine levels in the
212 organism (Stipanuk et al. 2009), especially in strict carnivores like the gyrfalcon. *CTNS*
213 expression was highest in gyrfalcon nestlings reared in territories with highest food
214 abundance, meaning that this gene is epigenetically labile in gyrfalcons. We thus suggest
215 that epigenetic sensitivity to protein intake in *CTNS* may contribute to cysteine
216 homeostasis, because a high expression of this gene avoids cystine accumulation in
217 melanosomes (Chiaverini et al. 2012).

218 *CTNS* affects pheomelanin synthesis, whose induction may represent a mechanism
219 to remove excess cysteine (Galván et al. 2012). However, *CTNS* only affects
220 pheomelanogenesis under high systemic cysteine levels in the organism as derived from a
221 comparison between levels of cystine in mouse blood (50 μ M) and in B16 cell culture
222 medium (380 μ M), suggesting the existence of a threshold cysteine/cystine concentration
223 below which cystinosis activity and pigmentation are unaffected (Chiaverini et al. 2012).
224 While the threshold value will have to be investigated in birds, this may explain why *CTNS*
225 expression increased and not decreased with food abundance in gyrfalcons. The possible
226 physiological benefits derived from the epigenetic lability of *CTNS* may therefore be
227 related to cysteine efflux instead of pheomelanin synthesis. This may thus contribute to
228 cysteine homeostasis without affecting pigmentation. In fact, although we could not
229 characterize the plumage pigmentation of nestling gyrfalcons, it is known that a single
230 plumage color variant that is explained by certain *MC1R* alleles exists in the Icelandic
231 population of this species (Johnson et al. 2012). In other words, Icelandic gyrfalcons show
232 variation in the expression of *CTNS* in feather melanocytes according to food abundance
233 in the breeding territories, but little variation in plumage pigmentation.

234 The expression of other genes that regulate pheomelanogenesis by influencing
235 cysteine availability (*Slc7a11* and *Slc45a2*) were not affected by food abundance.
236 Interestingly, however, *Slc7a11* can be downregulated under environmental conditions
237 that generate oxidative stress (Conrad and Sato 2012; Galván et al. 2017). Therefore,
238 epigenetic lability in *Slc7a11* and *CTNS* may have evolved because of the physiological
239 benefits of modulating their expression according to the double-sided nature of
240 pheomelanin, which depends on environmental conditions (Galván et al. 2012; Fig. 3).
241 Thus, conditions generating oxidative stress may downregulate *Slc7a11* expression, which
242 avoids cysteine transport to melanocytes, while conditions generating excess cysteine
243 may upregulate *CTNS* expression, which avoids cysteine accumulation in melanosomes.
244 Given the intrinsic association between these processes and pheomelanin synthesis, the
245 pigmentation phenotype of animals may also be affected in some cases (Galván et al.
246 2017), and either oxidative stress or excess cysteine derived from protein intake would
247 lead to an inhibition of the expression of pheomelanin-based pigmentation (Fig. 3). As
248 melanin-based pigmentation contributes to several adaptive benefits, ranging from
249 protection against mutagenic ultraviolet (UV) radiation to visual communication (Galván
250 and Solano 2016), it is expected that the evolution of pigmentation phenotypes closely
251 interacts with the physiological need of animals for modulating *Slc7a11* and *CTNS*
252 expression in response to environmental factors.

253 However, it is not expected that all species have evolved the capacity to adjust
254 *CTNS* expression to dietary influences. In this regard, *CTNS* dysfunction causes cystinosis
255 in humans and other animals, an autosomal recessive disease that has renal failure as the
256 most severe symptom (Chiaverini et al. 2012). A specific gene therapy has proved
257 successful at decreasing cystine levels in *CTNS*-deficient cells in mice (Harrison et al.
258 2013). This response would possibly be enhanced by an additional inducement of *CTNS*
259 expression, which may be achieved by a mechanism similar to that allowing gyrfalcons to

260 increase *CTNS* expression in response to food abundance. Although to our knowledge no
261 study has addressed the possibility that exposure to solar (UV) radiation affects the
262 expression of pigmentation genes in birds, this is known to occur in humans regarding the
263 *MC1R* gene (Scott et al. 2002), thus the potential influence of UV exposure on *CTNS*
264 should be considered together with diet. Future studies should investigate this potential
265 applicability of *CTNS* epigenetic lability to avoid the physiological consequences of
266 cystinosis in animals including humans.

267 It could also be suggested that the potential lack of dietary influence on *CTNS*
268 expression in humans may represent a physiological cost for those producing large
269 amounts of pheomelanin (i.e. associated to low *CTNS* expression; Chiaverini et al. 2012)
270 such as humans with the red hair phenotype (Ito et al. 2011), thus contributing to some
271 damaging consequences associated to this phenotype like a higher resistance to
272 analgesics and an increased tendency to bruising (Liem et al. 2006). Some evidence
273 suggests that Neanderthal hominids had a red hair phenotype (Lalueza-Fox et al. 2007),
274 and it is known that their diet was heavily based on meat in at least some populations
275 (Weyrich et al. 2017), thus allowing to speculate that a low *CTNS* expression with no
276 epigenetic lability may have contributed to their extinction. Our study should stimulate
277 research on the new field on the interaction between epigenetic lability and pigmentation
278 phenotype in a variety of study areas.

279

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287

288 **Conflict of Interest:** The authors declare that they have no conflict of interest.

289

290 **Statement of Animal Rights:** All applicable institutional and/or national guidelines for the
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292

293 **References**

294 Bellomo F, Corallini S, Pastore A, Palma A, Laurenzi C, Emma F, Taranta A (2010)
295 Modulation of CTNS gene expression by intracellular thiols. Free Rad Biol
296 Med 48:865-872.

297 Chiaverini C, Sillard L, Flori E, Ito S, Briganti S, Wakamatsu K, Fontas E, Berard E,
298 Cailliez M, Cochat P, et al. (2012) Cystinosin is a melanosomal protein that
299 regulates melanin synthesis. FASEB J 26:3779-3789.

300 Chintala S, Li W, Lamoreux ML, Ito S, Wakamatsu, K, Sviderskaya EV, Bennett DC, Park
301 YM, Gahl WA, Huizing M et al. (2005) *Slc7a11* gene controls production of
302 pheomelanin pigment and proliferation of cultured cells. Proc Natl Acad Sci USA
303 102:10964-10969.

304 Conrad M, Sato H (2012) The oxidative stress-inducible cystine/glutamate antiporter,
305 system x_c^- : cystine supplier and beyond. Amino Acids 42:231-246.

306 Galván I, Jorge A (2015) The rusty plumage coloration of juvenile gyrfalcons is produced
307 by pheomelanin and its expression is affected by an intracellular antioxidant. J
308 Raptor Res 49:59-65.

309 Galván I, Solano F (2016) Bird integumentary melanins: biosynthesis, forms, function and
310 evolution. Int J Mol Sci 17:520.

- 311 Galván I, Mousseau TA, Møller AP (2011) Bird population declines due to radiation
312 exposure at Chernobyl are stronger in species with pheomelanin-based
313 coloration. *Oecologia* 165:827-835.
- 314 Galván I, Ghanem G, Møller AP (2012) Has removal of excess cysteine led to the
315 evolution of pheomelanin? *BioEssays* 34:565-568.
- 316 Galván I, Bonisoli-Alquati A, Jenkinson S, Ghanem G, Wakamatsu K, Mousseau TA,
317 Møller AP (2014) Chronic exposure to low-dose radiation at Chernobyl favours
318 adaptation to oxidative stress in birds. *Funct Ecol* 28:1387-1403.
- 319 Galván I, Inácio A, Romero-Haro AA, Alonso-Alvarez C (2017) Adaptive downregulation of
320 pheomelanin-related *Slc7a11* gene expression by environmentally induced
321 oxidative stress. *Mol Ecol* 26:849-858.
- 322 García-Borrón JC, Olivares Sánchez MC (2011) Biosynthesis of melanins. In: Borovanský
323 J, Riley PA (eds) *Melanins and melanosomes: biosynthesis, biogenesis,*
324 *physiological, and pathological functions.* Wiley-Blackwell, Weinheim, pp 87-116.
- 325 Harrison F, Yeagy BA, Rocca CJ, Kohn DB, Salomon DR, Cherqui S (2013)
326 Hematopoietic stem cell gene therapy for the multisystemic lysosomal storage
327 disorder cystinosis. *Mol Ther* 21:433-444.
- 328 Hearing VJ. Genetics of melanosome structure and function. In: Borovanský J, Riley PA
329 (eds) *Melanins and melanosomes: biosynthesis, biogenesis, physiological, and*
330 *pathological functions.* Wiley-Blackwell, Weinheim, pp 323-341.
- 331 Hubbard JK, Uy JAC, Hauber ME, Hoekstra HE, Safran RJ (2010) Vertebrate
332 pigmentation: from underlying genes to adaptive function. *Trends Genet* 26:231-
333 239.
- 334 Ito S, Nakanishi Y, Valenzuela RK, Brilliant MH, Kolbe L, Wakamatsu K (2011) Usefulness
335 of alkaline hydrogen peroxide oxidation to analyze eumelanin and pheomelanin in

- 336 various tissue samples: application to chemical analysis of human hair melanins.
337 Pigment Cell Melanoma Res 24:605-613.
- 338 Johnson JA, Ambers AD, Burnham KK (2012) Genetics of plumage color in the Gyrfalcon
339 (*Falco rusticolus*): analysis of the melanocortin-1 receptor gene. J Hered 103:315-
340 321.
- 341 Karell P, Ahola K, Karstinen T, Valkama J, Brommer JE (2011) Climate change drives
342 microevolution in a wild bird. Nat Commun 2:208.
- 343 Klasing KC (1998) Comparative avian nutrition. CAB International, Wallingford.
- 344 Lalueza-Fox C, Römpler H, Caramelli D, Stäubert C, Catalano G, Hughes D, Rohland N,
345 Pilli E, Longo L, Condemi S et al. (2007) A melanocortin 1 receptor allele suggests
346 varying pigmentation among Neanderthals. Science 318:1453-1455.
- 347 Liem EB, Hollensead SC, Joiner TV, Sessler DI (2006) Women with red hair report a
348 slightly increased rate of bruising but have normal coagulation tests. Anesth Analg
349 102:313-318.
- 350 Lin SJ, Foley J, Jiang TX, Yeh CY, Wu P, Foley A, Yen CM, Huang YC, Cheng HC, Chen
351 CF et al. (2013) Topology of feather melanocyte progenitor niche allows complex
352 pigment patterns to emerge. Science 340:1442-1445.
- 353 Mohanty S, Kumar A, Dhawan J, Sreenivas V, Gupta S (2011) Noncultured extracted hair
354 follicle outer root sheath cell suspension for transplantation in vitiligo. Brit J
355 Dermatol 164:1241-1246.
- 356 Nadeau NJ, Minvielle F, Ito S, Inoue-Murayaina M, Gourichon D, Follett SA, Burke T,
357 Mundy NI (2008) Characterization of Japanese quail yellow as a genomic deletion
358 upstream of the avian homolog of the mammalian *ASIP* (*agouti*) gene. Genetics
359 178:777-786.
- 360 Nielsen ÓK (2003) The impact of food availability on gyrfalcon (*Falco rusticolus*) diet and
361 timing of breeding. In: Thompson DBA, Redpath SM, Fielding AH, Marquiss M,

- 362 Galbraith CA (eds) Birds of prey in a changing environment. Scottish Natural
363 Heritage, Edinburgh, pp 283-302.
- 364 Panzella L, Leone L, Greco G, Vitiello G, D'errico G, Napolitano A, d'Ischia M (2014) Red
365 human hair pheomelanin is a potent pro-oxidant mediating UV-independent
366 contributory mechanisms of melanomagenesis. *Pigment Cell Melanoma*
367 *Res* 27:244-252.
- 368 Park S, Imlay JA (2003) High levels of intracellular cysteine promote oxidative DNA
369 damage by driving the Fenton reaction. *J Bacteriol* 185:1942-1950.
- 370 R Core Team (2017) R: a language and environment for statistical computing. R
371 Foundation for Statistical Computing, Vienna.
- 372 Robert SM, Buckingham SC, Campbell SL, Robel S, Holt KT, Ogunrinu-Babarinde T,
373 Warren PP, White DM, Reid MA, Eschbacher JM, et al. (2015) SLC7A11
374 expression is associated with seizures and predicts poor survival in patients with
375 malignant glioma. *Sci Transl Med* 7:289ra86.
- 376 Scott MC, Suzuki I, Abdel-Malek ZA (2002) Regulation of the human melanocortin 1
377 receptor expression in epidermal melanocytes by paracrine and endocrine factors
378 and by ultraviolet radiation. *Pigment Cell Melanoma Res* 15:433-439.
- 379 Stipanuk MH, Dominy JE Jr, Lee J-I, Coloso RM (2006) Mammalian cysteine metabolism:
380 new insights into regulation of cysteine metabolism. *J Nutr* 136:1652S-1659S.
- 381 Stipanuk MH, Ueki I, Dominy JE, Jr, Simmons CR, Hirschberger LL (2009) Cysteine
382 dioxygenase: a robust system for regulation of cellular cysteine levels. *Amino Acids*
383 37:55-63.
- 384 Viña J, Saez GT, Wiggins D, Roberts AFC, Hems R, Krebs HA (1983) The effect of
385 cysteine oxidation on isolated hepatocytes. *Biochem J* 212:39-44.

386 Wang H, Osseiran S, Igras V, Nichols AJ, Roider EM, Pruessner J, Tsao H, Fisher DE,
387 Evans CL (2016) *In vivo* coherent Raman imaging of the melanomagenesis-
388 associated pigment pheomelanin. Sci Rep 6:37986.

389 Weyrich LS, Duchene S, Soubrier J, Arriola L, Llamas B, Breen J, Morris AG, Alt KW,
390 Caramelli D, Dresely V et al. (2017) Neanderthal behaviour, diet, and disease
391 inferred from ancient DNA in dental calculus. Nature 544:357-361.

392 Xu X, Dong GX, Hu XS, Miao L, Zhang XL, Zhang DL, Yang HD, Zhang TY, Zou ZT,
393 Zhang TT, et al. (2013) The genetic basis of white tigers. Curr Biol 23:1031-1035.

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412 **Fig. 1** Gyrfalcon nestlings in a nest surrounded by prey remains in Iceland (credit: Daniel
413 Bergmann). Insert photograph shows details of the melanin-based pigment pattern of tail
414 feathers.

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416 **Fig. 2** Relationship between *CTNS* expression in feather melanocytes of gyrfalcon
417 nestlings and two estimates of food abundance in the territories where they were reared:
418 number of prey (A) and biomass of prey (B). Lower C_t values indicate higher gene
419 expression levels. The lines are the best-fit lines.

420

421 **Fig. 3** Schematic representation of a melanocyte with potentially adaptive epigenetic
422 lability in response to two environmental factors. On one hand, protein intake can increase
423 systemic levels of cysteine, thus potentially increasing its levels inside melanocytes. The
424 gene *CTNS* can be upregulated in response to this, pumping cysteine out of melanosomes
425 (yellow granules) and thus avoiding its accumulation. As pheomelanin synthesis takes
426 place inside pheomelanosomes, *CTNS* upregulation can also inhibit pheomelanin-based
427 pigmentation in skin and associated structures such as feathers and hairs, although this
428 inhibition only occurs above a threshold of systemic cysteine concentration. On the other
429 hand, oxidative stress produced by environmental factors can induce the downregulation
430 of the gene *Slc7a11*, thus decreasing the transport of cysteine within melanocytes and the
431 availability of this amino acid to pheomelanosomes, again inhibiting the expression of
432 pheomelanin-based pigmentation.

**PHEOMELANIN-BASED
PIGMENTATION**

**PHEOMELANIN-BASED
PIGMENTATION**

GROWING FEATHERS

**OXIDATIVE
STRESS**

Slc7a11

- CYS

+ CYS

Nucleus

**CTNS
+**

**PROTEIN
INTAKE**