Transcriptomic data analysis and differential gene expression of antioxidant pathways in king penguin juveniles (*Aptenodytes patagonicus*) before and after acclimatization to marine life

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In this article, we present differentially expressed gene profiles in the pectoralis muscle of wild juvenile king penguins that were either naturally acclimated to cold marine environment or experimentally immersed in cold water as compared with penguin juveniles that never experienced cold water immersion. Transcriptomic data were obtained by hybridizing penguins total cDNA on Affymetrix GeneChip Chicken Genome arrays and analyzed using maxRS algorithm, “Transcriptome analysis in non-model species: a new method for the analysis of heterologous hybridization on microarrays” (Dégletagne et al., 2010) [1]. We focused on genes involved in multiple antioxidant pathways. For better clarity, these differentially expressed genes were clustered into six functional groups according to their role in controlling redox homeostasis. The data are related to a comprehensive research study on the ontogeny of antioxidant functions in king penguins, “Hormetic response triggers multifaceted anti-oxidant strategies in immature king penguins (*Aptenodytes patagonicus*)” (Rey et al., 2016) [2]. The raw
microarray dataset supporting the present analyses has been deposited at the Gene Expression Omnibus (GEO) repository under accessions GEO: GSE17725 and GEO: GSE82344.

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### Specifications Table

| Subject area               | Biology                                                                 |
|----------------------------|-------------------------------------------------------------------------|
| More specific subject area | Oxidative stress physiology                                            |
| Type of data               | Table                                                                   |
| How data was acquired      | Microarray data were obtained by DNA microarray hybridization (Affymetrix GeneChip® Chicken Genome Array). Tissue: pectoralis muscle biopsy of juvenile king penguins excised under general anesthesia. |
| Data format                | Analyzed, raw data                                                      |
| Experimental factors       | Total RNA was extracted from pectoralis muscle; biotin labeling and hybridization were performed following standard Affymetrix protocol. |
| Experimental features      | Never-immersed juvenile penguins serve as control and were compared i) to naturally acclimated penguins returning from a foraging trip at sea and ii) to naïve penguins artificially acclimated to cold water by repeated immersions. |
| Data source location       | Port Alfred, Possession Island (Crozet Archipelago, 46°25’ S, 51°45’ E) and Lyon University (France). |
| Data accessibility         | Data is within this article and raw data is available in Gene Expression Omnibus repositories (GEO: GSE17725; http://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE17725 and GEO: GSE82344; http://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?token=yxibwieatzavpqv&acc=GSE82344). |

### Value of the data

- Our transcriptomic analysis of gene expression in the pectoralis muscle of wild juveniles king penguin allows the detection of multiple antioxidant pathways.
- These data provided evidences that an activation of powerful and coordinated antioxidant strategies occurs in the pectoralis muscle of king penguin juveniles during the transition from a terrestrial to a marine life style.
- These original results can serve as a reference point for various studies related to the mechanisms controlling redox homeostasis in natural populations for which data availability remains scarce and usually restricted to the detection of few antioxidant molecules.

### 1. Data

Here, we provide the expression profile of gene involved in the control of redox homeostasis in the pectoralis muscle of three groups of king penguin juveniles (Aptenodytes patagonicus) differing in their degree of acclimation to marine environment. Targeted genes are clustered into six groups as follow: the genes encoding proteins involved in non-mitochondrial ROS generation (Cluster 1), antioxidat enzymes (cluster 2), heat choc and chaperone proteins (Cluster 3), DNA repairs processes (Cluster 4), repair or degradation of damaged proteins (Cluster 5) and lipid membrane composition.
Table 1
Microarray data analysis centered on the genes encoding proteins involved in the regulation of the redox homeostasis.

| Symbol | Name | PPsSets | log2 (SA/NI) % | SA/NI % | P-value | log2 (AA/NI) % | AA/NI % | P-value |
|--------|------|---------|----------------|--------|---------|----------------|--------|---------|
| ANGPTL4 | angiopoietin-like 4 | GgaAffx.395.1. | −0.31 | −19% | 0.033 | | | ns |
| AOX1 | aldehyde oxidase 1 | GgaAffx.5165.3. | −0.49 | −29% | 0.031 | −0.49 | −29% | 0.031 |
| AOX2 | aldehyde oxidase 2 | GgaAffx.5165.4. | 0.68 | 61% | 0.003 | 0.42 | 34% | 0.037 |
| DUOX2 | dual oxidase 2 | GgaAffx.1631.1. | −0.35 | −22% | 0.029 | | | |
| DUOX1 | dual oxidase maturation factor 1 | GgaAffx.1645.3. | 0.28 | 22% | 0.017 | | | ns |
| NOX1 | NADPH oxidase 1 | GgaAffx.22036.3. | −0.3 | −19% | 0.042 | | | ns |
| PXDN | peroxidin homolog | Gga.14999.1. | −1.2 | 56% | 0.004 | −0.94 | −48% |
| SCARF1 | scavenger receptor class F. member 1 | Gga.7260.2. | −0.73 | −40% | 0.000 | −0.42 | −25% | 0.014 |
| SIRT1 | sirtuin 1 | GgaAffx.1802.1. | 0.48 | 39% | 0.050 | | | ns |
| SIRT5 | sirtuin 5 | Gga.12456.1. | 0.82 | 77% | 0.002 | | | |
| SIRT6 | sirtuin 6 | GgaAffx.23594.1. | 0.36 | 28% | 0.042 | | | |
| TNFRSF11A | tumor necrosis factor receptor superfamily. member 11a NFKB activator | GgaAffx.8155.1. | −0.42 | −26% | 0.024 | | | ns |
| TNFRSF18 | tumor necrosis factor receptor superfamily. member 18 | GgaAffx.11426.1. | −0.51 | −30% | 0.007 | | | ns |
| TNFRSF21 | tumor necrosis factor receptor superfamily. member 21 | Gga.4943.1.S1_at | −1.16 | −55% | 0.012 | −0.79 | −43% | 0.045 |

Cluster 2: Genes encoding antioxidant enzymes

| Symbol | Name | PPsSets | log2 (SA/NI) % | SA/NI % | P-value | log2 (AA/NI) % | AA/NI % | P-value |
|--------|------|---------|----------------|--------|---------|----------------|--------|---------|
| BLVRA | biliverdin reductase A | GgaAffx.23872.1. | 0.51 | 42% | 0.039 | | | ns |
| GPX4 | glutathione peroxidases | Gga.107.1.S1_at | 0.94 | 92% | 0.000 | | | ns |
| HMOX1 | heme oxygenase 1 | Gga.2039.1.S1_at | 1.39 | 162% | 0.050 | 2.17 | 350% | 0.006 |
| HMOX2 | heme oxygenase (decycling) 2 | Gga.9310.1. | −0.57 | −33% | 0.003 | | | ns |
| MGST3 | microsomal glutathione S-transferase 3 | Gga.7258.1.S1_at | 0.6 | 52% | 0.010 | | | ns |
| MT2A | metallothionein 2A | Gga.4210.1.S1_at | 2.06 | 316% | 0.001 | 1.66 | 217% | 0.004 |
| MT3 | metallothionein 3 | GgaAffx.9262.1. | 1.48 | 180% | 0.005 | 1.44 | 171% | 0.006 |
| PRDX3 | peroxiredoxin 3 | Gga.4515.3. | 0.42 | 34% | 0.015 | | | ns |
| SOD1 | superoxide dismutase 1 | Gga.3346.1. | 0.42 | 34% | 0.025 | | | ns |
| TXNDC10 | thioredoxin domain containing 10 | Gga.17473.1. | −0.96 | −49% | 0.000 | −0.71 | −39% | 0.001 |

Cluster 3: Genes encoding heat shock or chaperone proteins

| Symbol | Name | PPsSets | log2 (SA/NI) % | SA/NI % | P-value | log2 (AA/NI) % | AA/NI % | P-value |
|--------|------|---------|----------------|--------|---------|----------------|--------|---------|
| HSF3 | heat shock factor 3 | Gga.5116.3. | 0.33 | 26% | 0.023 | | | ns |
| HSF4 | heat shock transcription factor 4 | GgaAffx.2032.2. | 0.45 | 36% | 0.022 | | | |

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| Symbol | Name | PPSets | log2 (SA/NI) | SA/NI % | P-value | log2 (AA/NI) | AA/NI % | P-value |
|--------|------|--------|--------------|---------|---------|--------------|---------|---------|
| CRYAA  | crystallin. alpha A | GgaAffx.10353.1. S1_at | 0.39 | 31% | 0.027 | ns |
| CRYAB  | crystallin. alpha B | Gga.1999.1. S1_a_at | 0.96 | 95% | 0.021 | ns |
| HSPE1  | heat shock 10 kDa protein 1 | Gga.4873.1. S1_a_at | −0.55 | −32% | 0.002 | −0.33 | −20% | 0.039 |
| HSPB1  | heat shock 27 kDa protein 1 | Gga.1809.1. S1_at | −0.45 | −27% | 0.008 | ns |
| HSPB7  | heat shock 27 kDa protein family. member 7 | Gga.11398.1. S1_at | 0.95 | 93% | 0.000 | ns |
| HSPD1  | heat shock 60 kDa protein 1 | Gga.9897.1. S1_a_at | −0.75 | −41% | 0.000 | −0.86 | −45% | 0.000 |
| DNAJA4 | DnaJ (Hsp40) homolog. subfamily A. member 4 | Gga.1999.1. S1_a_at | 0.96 | 95% | 0.021 | ns |
| DNAJ8  | DnaJ (Hsp40) homolog. subfamily B. member 9 | Gga.12760.1. S1_a_at | −0.87 | −45% | 0.019 | −1.10 | −53% | 0.005 |
| DNAJC6 | DnaJ (Hsp40) homolog. subfamily C. member 6 similar to heat shock protein 67B2 | Gga.23432.1. S1_a_at | −0.48 | −28% | 0.004 | ns |
| HSP67B2| heat shock protein 67B2 | Gga.16163.1. S1_a_at | 1.38 | 160% | 0.000 | ns |
| HSPA14 | heat shock 70 kDa protein 14 | Gga.19503.1. S1_at | −0.61 | −34% | 0.001 | −0.44 | −27% | 0.011 |
| HSPA8  | heat shock 70 kDa protein 8 | Gga.4555.1. S1_at | −0.71 | −39% | 0.003 | ns |

**Cluster 4: Genes encoding proteins involved in DNA repair processes**

| Symbol | Name | PPSets | log2 (SA/NI) | SA/NI % | P-value | log2 (AA/NI) | AA/NI % | P-value |
|--------|------|--------|--------------|---------|---------|--------------|---------|---------|
| PARP6  | poly (ADP-ribose) polymerase family. member 6 | Gga.1599.1. S1_a_at | 0.29 | 22% | 0.045 | ns |
| PARP8  | poly (ADP-ribose) polymerase family. member 8 | Gga.24537.1. S1_a_at | 0.31 | 24% | 0.040 | 0.34 | 27% | 0.024 |
| PARP16 | poly (ADP-ribose) polymerase family. member 16 | Gga.8044.1. S1_at | 0.43 | 35% | 0.037 | ns |
| XRCC2  | X-ray repair complementing defective repair cells 2 | Gga.12290.1. S1_at | −0.55 | −32% | 0.003 | ns |
| XRCC4  | X-ray repair complementing defective repair cells 4 | Gga.24733.1. S1_a_at | 0.29 | 22% | 0.025 | ns |
| ERCC4  | excision repair cross-complementing group 4 | Gga.12489.1. A1_at | 0.54 | 45% | 0.032 | ns |
| RAD21L1| RAD21-like 1 | Gga.3857.1. S1_at | 0.45 | 37% | 0.022 | ns |
| RAD51L3| RAD51-like 3 | Gga.9680.1. S1_x_at | 0.29 | 22% | 0.035 | ns |
| RAD23B | RAD23 homolog B | Gga.1359.1. S1_at | 0.31 | 24% | 0.037 | 0.43 | 34% | 0.008 |
| DDB1   | damage-specific DNA binding protein 1. 127 kDa | Gga.5146.1. S1_at | 0.49 | 40% | 0.007 | ns |
| DDB2   | damage-specific DNA binding protein 2. 48 kDa | Gga.12520.1. S1_a_at | 0.27 | 21% | 0.048 | 0.31 | 24% | 0.031 |
| POLCD  | polymerase (DNA directed). epsilon 3 | Gga.4785.1. S1_at | −0.62 | −35% | 0.006 | ns |
| RFC1   | replication factor C (activator 1) 1. 145 kDa | Gga.6031.1. S1_a_at | −0.91 | −89% | 0.000 | ns |
| UNG    | uracil-DNA glycosylase methyl-CpG binding domain protein 4 | Gga.4682.1. S1_at | −0.42 | −25% | 0.036 | −0.43 | −26% | 0.033 |
## Table 1 (continued)

| Symbol | Name                                                                 | PPSets                        | log2 (SA/NI) | SA/NI % | P-value   | log2 (AA/NI) | AA/NI % | P-value |
|--------|---------------------------------------------------------------------|-------------------------------|--------------|---------|-----------|--------------|---------|---------|
| MSRA   | methionine sulfoxide reductase A                                     | GgaAffx.25021.1. S1_s_at      | 0.65         | 57%     | 0.001     | ns           |         |
| PSM7   | proteasome subunit. alpha type. 7                                   | Gga.2045.2. S1_a_at           | 0.58         | 49%     | 0.006     | 0.58         | 49%     | 0.006   |
| PSM8   | proteasome subunit. beta type. 1                                    | Gga.4653.2. S1_a_at           | 0.38         | 31%     | 0.043     | ns           |         |
| PSM3   | proteasome subunit. beta type. 3                                    | Gga.1459.1.S1_at              | 0.52         | 43%     | 0.000     | 0.71         | 63%     | 0.000   |
| PSMC3  | proteasome 26S subunit. ATPase. 3                                   | Gga.4649.1. S1_s_at           | 0.36         | 28%     | 0.008     | ns           |         |
| PSMC6  | proteasome 26S subunit. ATPase. 6                                   | Gga.16005.1. S1_s_at          | 0.6          | 52%     | 0.032     | ns           |         |
| PSM4   | proteasome 26S subunit. non-ATPase. 4                               | Gga.6030.1. S1_s_at           | 0.33         | 26%     | 0.010     | ns           |         |
| PSME3  | proteasome activator subunit 3                                      | Gga.5999.2. S1_at             | −0.42        | −25%    | 0.021     | ns           |         |
| –      | proteasome C1 subunit                                               | GgaAffx.8554.1. S1_x_at       | 0.33         | 26%     | 0.025     | 0.45         | 37%     | 0.005   |
| POMP   | proteasome maturation protein                                       | Gga.5765.1.S1_at              | 0.32         | 25%     | 0.020     | 0.44         | 36%     | 0.003   |
| SMURF1 | SMAD specific E3 ubiquitin protein ligase 1                         | GgaAffx.2883.1. S1_s_at       | 0.81         | 75%     | 0.011     | ns           |         |
| UBB    | ubiquitin B                                                         | Gga.2501.2.S1_at              | 0.41         | 33%     | 0.023     | ns           |         |
| UBE2G2 | ubiquitin-conjugating enzyme E2G 2                                  | Gga.19739.1. S1_at            | 0.38         | 30%     | 0.003     | ns           |         |
| UBE4B  | ubiquitination factor E4B                                           | GgaAffx.25563.1. S1_s_at      | 0.39         | 31%     | 0.014     | ns           |         |
| UCHL1  | ubiquitin carboxyl-terminal esterase L1                              | Gga.9618.1.S1_at              | 2.22         | 366%    | 0.000     | 1.84         | 258%    | 0.000   |
| UCHL5  | ubiquitin carboxyl-terminal hydrolase L5                             | GgaAffx.12236.1. S1_s_at      | 0.61         | 52%     | 0.029     | ns           |         |
| UFD1L  | ubiquitin fusion degradation 1 like ubiquitin interaction motif con- | Gga.3094.1.S1_at              | 0.44         | 36%     | 0.010     | ns           |         |
| UIMC1  | containing 1                                                        | GgaAffx.768.2. S1_at          | 0.67         | 59%     | 0.001     | ns           |         |
| WWF1   | WW domain containing E3 ubiquitin protein ligase 1                  | GgaAffx.24796.1. S1_at        | 0.45         | 37%     | 0.022     | ns           |         |
| LONP2  | lon peptidase 2. peroxisomal                                        | Gga.12947.1. S1_s_at          | 0.68         | 60%     | 0.000     | ns           |         |
| LONRF1 | LON peptidase N-terminal domain and ring finger 1                   | GgaAffx.8741.1. S1_at         | 0.31         | 24%     | 0.041     | ns           |         |
| ATXN3  | ataxin 3                                                            | Gga.12408.1. S2_at            | −0.85        | −44%    | 0.000     | −0.48        | −29%    | 0.016   |
| NBR1   | neighbor of BRCA1 gene 1                                            | Gga.9984.1. S1_s_at           | 0.43         | 34%     | 0.013     | ns           |         |

### Cluster 5: Genes encoding proteins involved in repair or degradation of damaged proteins

Differentially expressed genes are presented as percentage change of never-immersed (NI) controls versus naturally acclimated to cold marine environment (sea acclimated: SA) or experimentally immersed in cold water (artificially acclimated: AA). For each gene, we provided its symbol followed by its common name and the Affymetrix ProbeSet identification number used to measure its expression. Genes were considered significantly differentially expressed when \( p \)-value < 0.05.
remodeling (Cluster 6). For each gene we provide its symbol, its name, the corresponding Affymetrix ProbeSet identification number and the percentage change of expression as compared to never-immersed control penguins.

2. Experimental design, materials and methods

2.1. Animals and sample collection

We captured king penguin juveniles of 10–11 month at the breeding colony of la Baie du Marin (Crozet Archipelago; French Southern Territories). A first group of penguins was held in an outdoor enclosure until they achieved their molt constituting the ‘never-immersed control’ group (NI, n = 4). A second group of penguins received the same treatment as the NI penguins but were subjected to repeated immersions in cold water (8 °C) over 3 weeks to simulate the acclimatization to marine life; this group is referred as artificially-acclimated penguins (AA, n = 3). NI penguins were also compared to juveniles of 12–14 month that returned from a foraging trip at sea and had fully accomplished their acclimatization to marine life (sea-acclimatized, SA, n = 3). We controlled for potential effect of nutritional status by feeding penguins with mackerel (Scomber vernalis) on a daily basis. At the end of the procedure, pectoralis muscle of each penguin was surgically biopsied under general anesthesia and the muscle biopsy was frozen at −80 °C. More details of the experimental procedure are given in Rey et al. [2].

2.2. RNA extraction

Total RNA was extracted following the single-step TriReagent protocol (Invitrogen, Cergy Pontoise, France). Briefly, 50 mg of pectoralis muscle was homogenized in 1 mL reagent with a Polytron homogenizer. The aqueous phase was transferred to a 2 mL Eppendorf tube containing 0.5 ml 2-propanol. Samples were incubated at room temperature for 5 min and subjected to a centrifugation at 12,000 g for 10 min at 4 °C. The pellet was washed twice with ethanol 75% and was re-suspended in ultra-pure water. The quality of extracted RNA (RNA integrity > 8) was assessed using a Bioanalyzer 2100 (Agilent technologies, Inc, Palto Alto, CA, USA).

2.3. Labeling and hybridization

Labeling and hybridization were performed on Affymetrix GeneChip® Chicken Genome Arrays by the ProfileXpert platform (Lyon, France) following the standard Affymetrix protocol (http://www.affymetrix.com), as described in Dégletagne et al. [1]. All arrays were scanned with a confocal laser (Genechip scanner 3000, Affymetrix).

2.4. Microarray analysis

We used the MaxRS method developed for the analysis of heterologous hybridization profiles [1], a method that has been previously applied in king penguins [3]. All results were normalized using the quantile method after log2 transformation to make them comparable across microarrays [4]. Gene expression of NI penguins, considered as control in the study, were compared to those of SA or AA groups. Differentially expressed genes between NI vs. SA or NI vs. AA were determined using the empirical Bayes moderated t-statistics implemented in the Bioconductor package limma [5]. We focused on the genes involved in the redox homeostasis and gathered them into six functional clusters according to GenOntology annotation and literature search [2,6,7]. All analyses were performed using the R statistical software Table 1.

Conflict of interest

None.
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Transparency document. Supporting information

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