1 Dataset Brief

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3 A proteome map of the pituitary melanotrope cell activated by black-background
4 adaptation of *Xenopus laevis*

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1 ABSTRACT

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3 Upon transfer of *Xenopus laevis* from a white to a black background, the
4 melanotrope cells in the pituitary pars intermedia secrete α -melanocyte-stimulating
5 hormone (α -MSH), which stimulates dispersion of melanin pigment in skin
6 melanophores. This adaptive behavior is under the control of neurotransmitters and
7 neuropeptides of hypothalamic origin. The α -MSH-producing cells and their
8 hypothalamic control system provide an interesting model to study proteins required
9 for biosynthetic and secretory processes involved in peptide hormone production and
10 for brain-pituitary signaling. We present a 2D-PAGE-based proteome map of
11 melanotrope cells from black-adapted animals, identifying 204 different proteins by
12 mass spectrometric analysis.

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16 Keywords : *Xenopus laevis*, 2D-PAGE, background adaptation, neuroendocrine cell

1 The hypothalamo-hypophyseal system of the amphibian *Xenopus laevis* represents
2 the complex plasticity of a neuroendocrine system involved in physiological
3 adaptation processes [1]. The system is triggered by various environmental stimuli
4 (light, temperature, stressors). The transduction process involves a number of
5 neurotransmitters/neuropeptides and two main secondary messengers, Ca^{2+} and
6 cAMP [2].

7 The size of the melanotrope cells increases dramatically upon stimulation,
8 concomitant with the increase in expression of the pro-opiomelanocortin (POMC)
9 gene, followed by massive production of the POMC-derived peptide hormone α -
10 melanocyte-stimulating hormone (α -MSH). α -MSH biosynthesis involves folding,
11 post-translational modifications, sorting, cleavage and packaging of POMC. Finally,
12 the secretion of α -MSH is performed by an exocytotic machinery. Expression of a
13 number of exocytosis-related gene products such as SNAP-25, munc-18, DOC2,
14 synaptobrevin and synaptotagmin has been shown to be stimulated by black-
15 background adaptation [2].

16 Here, we present a 2D-PAGE proteome map of the pars intermedia cells from black-
17 adapted toads (Fig.1). Mass spectrometry allowed the identification of the proteins
18 from all indicated spots, corresponding to 204 different proteins. All experimental
19 procedures are documented in Supporting materials S1. An overview of the data on
20 the identified spots is presented in the Supporting Table S1. The identified proteins
21 are listed in Table 1 according to their functional categories. We used the DAVID
22 bioinformatics tool for gene ontology analysis [3]. The results show that energy
23 metabolism dominates the biological process annotation and demonstrate that most
24 of the proteins have a specialized function in protein biosynthesis, processing and/or
25 secretion (Fig. 2). Besides energy metabolism, also ion transport and signal
26 transduction events are dominant processes in the highly activated melanotrope
27 cells. Concerning the molecular function of the identified proteins, a majority of the
28 proteins are classified as hydrolases, again indicating the importance of protein
29 processing and metabolic functions in melanotrope cells of black-background-
30 adapted animals. Below, we discuss the identified proteins with a role in POMC
31 folding, processing and secretory processes. Discussion on other relevant
32 processes, e.g. Ca^{2+} signalling, is presented in Supporting materials S2.

1 Given that adaptation for more than 3 weeks to a black background triggers the
2 animals to produce the precursor protein POMC and to release α -MSH thereby
3 stimulating melanin dispersion in skin melanophores, as expected, we detect POMC.
4 The spots 194-197 represent the A and B isoforms of POMC, but they do not
5 dominate the gel pattern, probably reflecting a high rate of proteolytic cleavage of
6 POMC. We also found POMC fragments of lower molecular weight (spots 294, 329
7 and 336-338), which are expressed above average intensity. The peptides identified
8 in these spots are located in the N-terminal portion of POMC and typically result from
9 cleavage of the POMC proprotein by prohormone convertase 1 (PC1, [4]). The C-
10 terminal POMC fragments are further processed via PC2, ultimately leading to the
11 formation of a number of peptides, including α -MSH. While PC1 is detected in two
12 distinct spots (spots 68, 70), PC2 is identified in multiple spots (53-62, 64), indicating
13 a more heterogeneous glycosylation, as reported [5]. Clearly, PC2 is more abundant
14 than PC1, confirming previous findings that the PC2 gene is more expressed in
15 melanotrope cells [6, 7]. Another relevant protein is 7B2 (spots 280, 283) which
16 modulates PC2 activity by acting as a molecular chaperone for PC2 folding, but
17 which is also an inhibitor of PC2 activity, preventing its premature activation [8,9].
18 Processing of the POMC-derived peptides, more specifically α -MSH, involves further
19 removal of the C-terminal basic dipeptide pair by carboxypeptidase E (spots 84,86).

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21 POMC is a glycoprotein and therefore requires the ER- and Golgi-residing protein
22 folding and processing machinery. Recently, we showed that melanotrope cells from
23 black-background-adapted animals display a high level of ER activities [10]. We now
24 confirm this by the identification of typical ER-resident chaperones in multiple
25 intensely coloured gel spots. The two known isoforms of calreticulin are both
26 expressed (spots 27-31 and 77). They nearly co-migrated on the 2D-PAGE gel but
27 could be distinguished via peptides containing the E213/D213 and D93/E93
28 polymorphisms. Other known ER-resident protein chaperones that were identified are
29 the protein disulfide isomerase A3 (Erp57, spots 105-113), glucose-regulated protein
30 GRP78 (BiP, spots 32-36 and 133), endoplasmic (spots 7-10), protein disulfide
31 isomerase A1 (Erp72, spots 79-81), and protein disulfide isomerase A6 (CaBP1/P5,
32 spots 133,135). Using comparative 2D-PAGE, Erp57, calreticulin and BiP are indeed
33 shown to be highly expressed in melanotropes from black adapted animals, reflecting

1 their role in protein biosynthesis [10]. The so-called ARMET protein (spots 317 and
2 319) has recently been associated with the unfolded protein response [11].
3 Furthermore, we observed the presence of the protein disulfide isomerase ERp46
4 (thioredoxin related protein 5), a poorly characterized protein previously identified in a
5 limited set of proteomic analyses (spots 143,166 [12]). The finding of two additional
6 thioredoxin-like Erp19 proteins (spots 310,326) completes the list of ER-based
7 chaperones. We also identified a protein (spots 25,26) highly similar to selenoprotein
8 M (Sep15), known as a protein involved in ER-based glycoprotein processing and
9 quality control [13], and homologous to the human protein kinase c substrate KH-80
10 [14].

11 α -MSH is stored as a processed peptide into large dense-core granules. Sorting into
12 these granules is mediated by carboxypeptidase E [15]. Regulated secretion of α -
13 MSH depends on the activation of voltage-dependent calcium channels [16, 17] and
14 involves the formation of a core complex which includes the SNARE proteins
15 syntaxin I, synaptobrevin and SNAP-25 implicated in membrane fusion [18]. Most
16 SNARE proteins are membrane bound and are not observed in this 2D-PAGE
17 analysis. However, we find several proteins that are linked to lipid rafts, including
18 flotillin-1 (spot 153), galectin-3 (Galactose-specific lectin 3, spot 216), V-
19 ATP synthase (spots 46,48,108-110) [19] and BASP-1 (spot 78) [20]. We also
20 identified Rab-35 (spots 244, 245), a protein previously observed in a proteomic
21 survey of adrenergic secretory vesicles [21], and a Rab GDI protein (spots 140,141),
22 which releases Rab proteins from the membrane.

23 Exocytosis requires a remodelling of the subplasmalemmal actin network [22].
24 Immature granules are transported in a microtubule-dependent manner to the actin-
25 rich cellular cortex where they mature. Partial depolymerisation of the actin is
26 necessary to empty the granules, allowing their recruitment at the plasma membrane.
27 Rho GTPases act as landmarks for membrane fusion by consecutive disassembly
28 and assembly of the actin. In this context, we discovered the Rho-GDP dissociation
29 inhibitor (spot 241). Apart from this regulatory protein, we found several proteins
30 known to be part of the actin network, including the WD repeat protein 1 (spot 76),
31 fascin (spot 150), tropomyosin (spots 223, 225, 228 and 230) and the F-capping
32 protein (spot 214). Transgelin (spot 286) is generally linked to smooth muscle tissue
33 but is clearly also present in the pars intermedia tissue. The proteins dynactin (spot

95) and spectrin (spot 1) are part of the dynactin/dynein complex which is involved in ER-to-Golgi transport [23].

In summary, our 2D-PAGE map represents a typical pattern for a strongly activated neuroendocrine cell with high levels of glycoprotein folding, processing and secretion, revealing a number of proteins that are now linked for the first time to prohormone biosynthesis. This dataset forms a resource that will be used for further investigation of this fascinating biological model system. We demonstrated the power of the method to discriminate isoforms and post-translationally modified versions.

Nevertheless, we are fully aware of the limitations of 2D-PAGE, e.g. in terms of the limited dynamic range and the poor resolution of transmembrane proteins. To gather more information on the signal transduction pathways, gel free and PTM-dedicated methods should be exploited for the same set of samples to provide more in-depth proteomic data.

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5 **Figure captions**
6

7 Fig. 1. A representative 2D-PAGE map of *X. laevis* intermediate pituitary proteins.
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9 Fig. 2. Gene ontology analysis of identified proteins. A. Summary of the classification
10 of the biological functions. B. Summary of the analysis of the molecular functions.
11

1 Fig.1

Fig. 2.A.

Fig.2.B.