## Melaninmisfarging i muskulatur hos laks



Erling Olaf Koppang Norges veterinærhøgskole Oslo

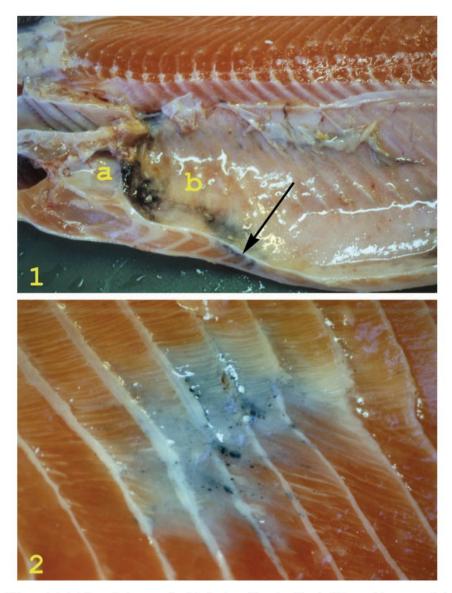


Figure 1 Gross pathological changes in the carcass of an Atlantic salmon. The pericardial cavity (a) is normal, but severe melanization is apparent in the abdominal cavity (b). Melanized musculature subjacent to the peritoneum is seen on the cut surface (arrow).

Figure 2 A melanized area in the musculature of an Atlantic salmon. The peritoneum is removed and darker foci are seen in a dark to grey area involving five myosepta. The lesion is situated laterally in the fish, covering the area of the lateral organ. Note the contraction in the musculature, disrupting the curves of the intramuscular septa.

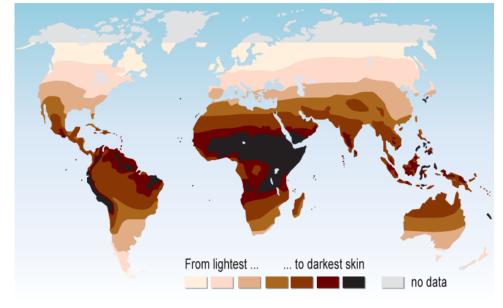






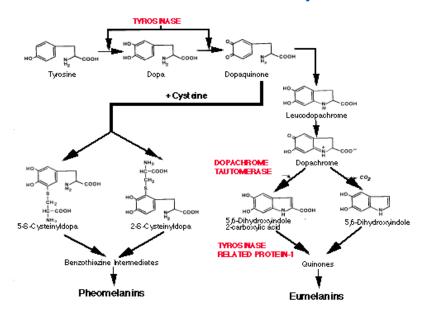




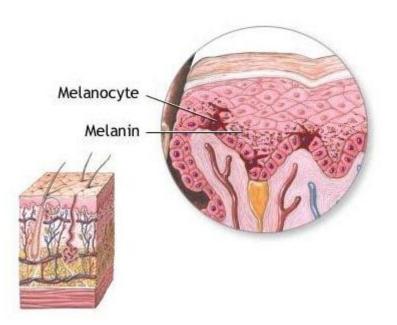


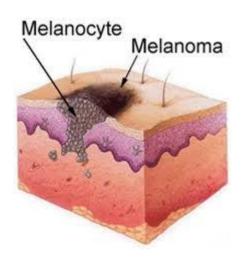
## 1. Hva er melanin?

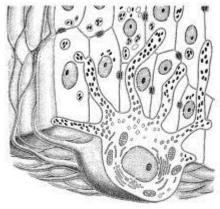
### The Melanin Chemical Pathway



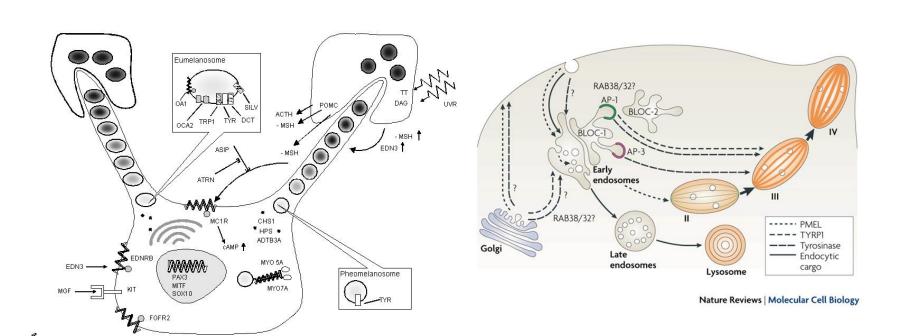
## Hvor dannes melanin?







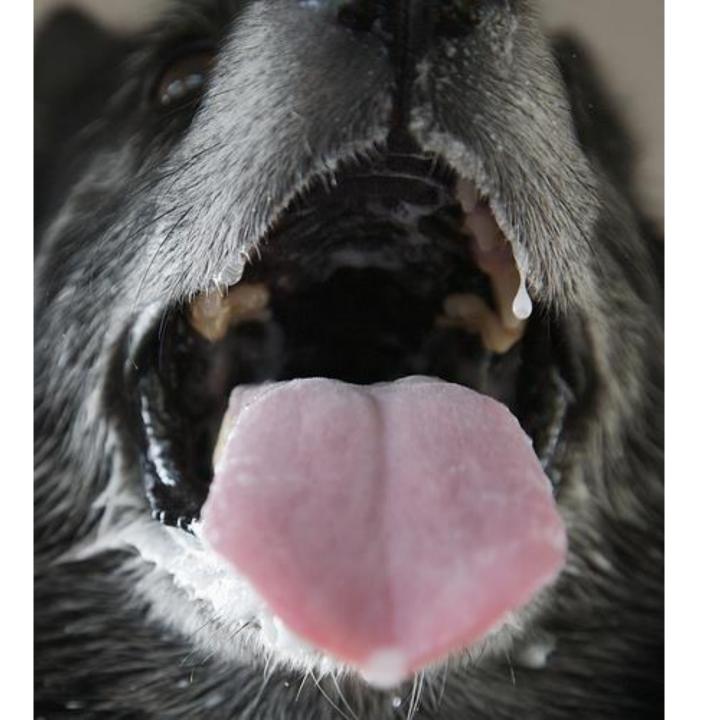
# Melanosom: den intracelluære melaninfabrikken



# 2. Hvilke funksjoner har melanin?









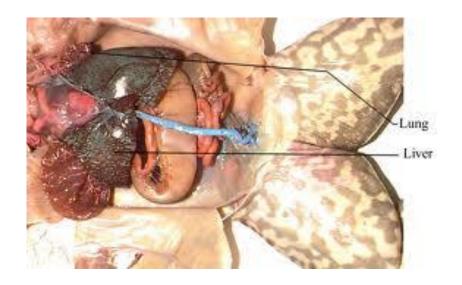




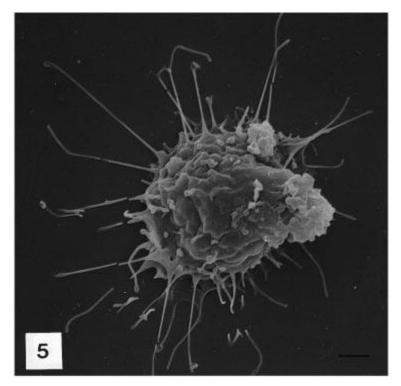
Giovanni Sichel – Universitetet i Catania

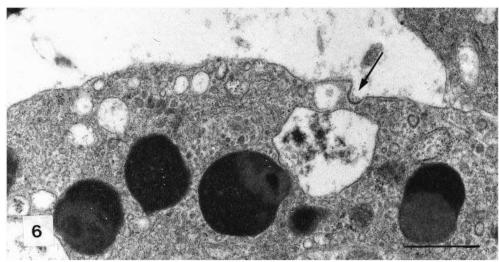












# Giovanni Sichel og medarbeidere:

- Melaninproduksjon hos virveldyr er ikke utelukkende relatert til celler av ectodermal opprinnelse
- Kupfferske celler i leveren hos amfibier kan lage melanin
- Kupfferske celler er av mesenchymal opprinnelse
- Altså: Populasjoner av forsvarsceller kan lage melanin – ulikt det man ser hos pattedyr

### Vaccine-associated granulomatous inflammation and melanin accumulation in Atlantic salmon, *Salmo salar* L., white muscle

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#### Abstrac

The purpose of this study was to investigate the nature of variably sized pigmented foci encountered in fillets of farmed Atlantic salmon, Salmo salar L. The material was sampled on the filler production line and on salmon farms from fish with an average size of 3 kg from various producers. The fish had been routinely vaccinated by injection. Gross pathology, histology, immunohistochemistry using antisera against major histocompatibility complex (MHC) class II β chain and transmission electron microscopy (TEM) were used to characterize the changes. Macroscopically, melanized foci were seen penetrating from the peritoneum deep into the abdominal wall, sometimes right through to the skin, and also embedded in the caudal musculature. Histological investigation revealed muscle degeneration and necrosis, fibrosis and granulomatous inflammation containing varying numbers of melano-macrophages. Vacuoles, either empty or containing heterogeneous material, were frequently seen. The presence of abundant MHC class II cells indicated an active inflammatory condition. TEM showed large extracellular vacuoles and leucocytes containing homogeneous material of lipid-like appearance. The results showed that the melanized foci in Atlantic salmon fillet resulted from an inflammatory condition probably induced by vaccination. The described condition is not known in wild salmon and in farmed salmon where injection vaccination is not applied.

Correspondence Trypre T Poppe, Department of Basic Sciences and Aquatic Medicine, Norwegian School of Veterinary Science, Ullevillisveien 72, Box 8146 Dep., 0033 Oslo, Norway (e-mail: trypre, poppe@veths.no) Keywords: Atlantic salmon, inflammation, melanomacrophage, major histocompatibility complex class II, mineral oil, vaccine.

#### . . . .

Various pathological conditions may be associated with abnormal prigeneution in tissues and organs. Such pigments may either be of congrous or endogenous origin. Endogenous originers include derivates of lipids, hacemoglobin, porphyrims and medianir. The term endonois is used to describe the presence of medanin in abnormal locations (Themson 1984). In werebrates, mediani is synthesized by medanocytes and organized in medanoms, which are hypomore-featured inracellular organells (Orlow 1995; Rapono, Fevrier, Stoornoms, with a 2002. Mammalian medanocytes originate from the embryonic neural rube (Sulisimon & Krifchell 2003) and it has been observed that such cells can migrate into inflamed tissue (Thomson 1984).

Inflammatory reactions and tissue regeneration in salmonids seen similar to those of mammals (Fina & Nielson 1971), but have in addition been associated with the involvement of so-called melano-macrophages (Roberts 1975; Agisus & Roberts 2003). The origins of melanosomes in melaniti-constaining viscerally located cells in fish is not clear (Agisus & Roberts 2003). but Sichel, Scalia, Mondia & Corasno (1997) suggested that melanogenesis in polidiohermic vertwhates may occur in mesenchyme-derived cells of the heamstopictic lineage, Although relocat melano-macrophages have been ascribed macrophage-like properties, their functions and significance are

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E O Koppang et al. Vaccine-associated pathology in salmon muscle



Figure 1 Gross pathological changes in the carcass of an Atlantic salmon. The pericardial cavity (a) is normal, but severe melanization is apparent in the abdominal cavity (b). Melanized musculature subjacent to the peritoneum is seen on the cut surface (arrow). Figure 2 A melanized area in the musculature of an Atlantic salmon. The peritoneum is removed and darker foci are seen in a dark to grey area involving five myosepta. The lesion is situated laterally in the fish, covering the area of the lateral organ. Note the contraction in the musculature, disrupting the curves of the intramuscular seption.

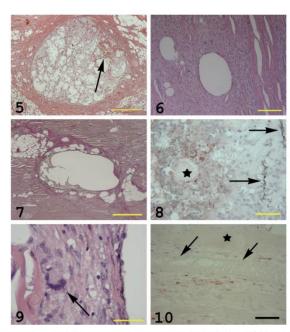


Figure 5 A large vesicle embedded in an intermyotomal septum containing macrophage-like cells, debris and a fresh haemorrhage (arrow) (H&E, bar = 500 µm).

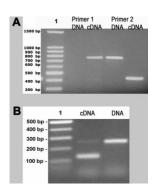
Figure 6 Empty vesicles surrounded by granulomatous tissue embedded in the white musculature. Note adjacent, seemingly unaffected muscle cells (H&E, bar =  $200 \ \mu m$ ).

Figure 7 Vesicles embedded in the white musculature surrounded by fibrogramulomatous tissue (red staining) (EVG, bar =  $500 \mu m$ ). Figure 8 Reaction against oil fred staining) in a vesicle as shown in Fig. 5. Homogeneous masses (astrisk) and macrophage-like cells show positive reactions. Note the melano-macrophage in the vesicle wall (arrows) (oil red O, bar =  $50 \mu m$ ).

Figure 9 High magnification of the wall of a vesicle as seen in Fig. 6. The wall contains a multinucleated giant cell (MGC)(arrow), epithelioid-like cells, small vacuoles and is lined towards the lumen of the greater vesicle with melanosome-containing cells, probably swollen melano-macrophages (H&E, bar = 40 µm).

Figure 10 Muscle cells infiltrated with MHC class II\* cells. One muscle cell is unaffected (asterisk). One fibre shows severe degeneration (arrowhead), whereas one is invaded by MHC class II\* cells (red reaction) (MHC class II immunostain, haematoxylin counterstain, bar = 100 μm).

Primer pairs recognising a fish CD83 homolog



 Primer pair detecting Dct/TRP-2 Copyright © 2006 The Authors Journal compilation © 2006 Blackwell Munksgaard

# Melanogenesis and evidence for melanosome transport to the plasma membrane in a CD83<sup>+</sup> teleost leukocyte cell line

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#### Summary

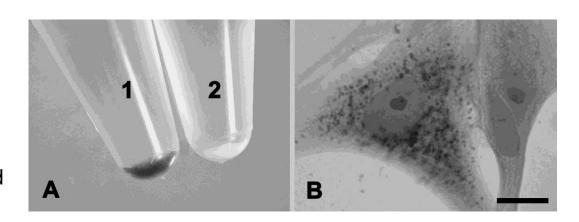
Key words: CD83/dendritic cell/endosomal pathway/ macrophage/melanogenesis/melanomacrophage/teleost

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#### Introduction

Melanins are complex polymeric pigments, which are formed by a wide variety of living organisms ranging from fungi and bacteria to higher vertebrates (Margalith, 1992; Orlow, 1995; Raposo et al., 2002). Common for

- A; 0,1 mM PTU inhibit tyrosinase dopachrome production from L-DOPA
- B; a few long cultured cells showed melanin reduction potential



### Isolation of the Atlantic salmon tyrosinase gene family reveals heterogenous transcripts in a leukocyte cell line

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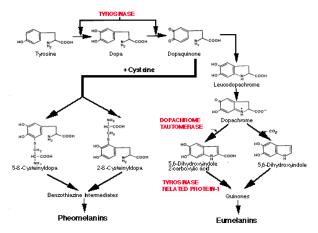
#### Summary

In ectothermic vertebrates, visceral organs harbor melanin-containing cells. Their ability as pigment producers is nevertheless disputed. To address expression of the key genes for melanogenesis in Atlantic salmon (Salmo salar), a tyrosinase-positive leukocyte cell line (SHK-1) and skin were used to obtain full-length tyrosinase (Tyr), tyrosinase-like protein-1 (Tyrp1), and dopachrome tautomerase (Dct) mRNA transcripts. In the SHK-1 cells, two different Tyrp1 transcripts were identified, one lacking exon 1. However, only the full-length version of Tyrp1 was identified in the skin. Sequen-

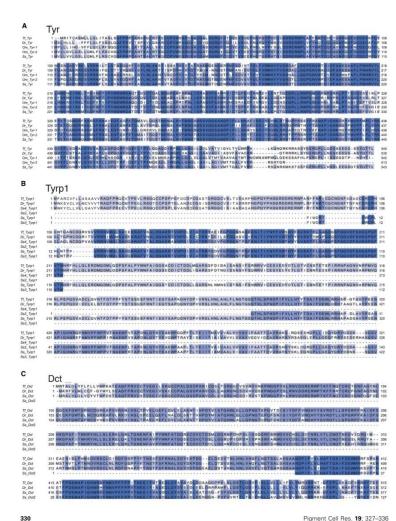
#### Introduction

The pigmentary system of vertebrates has predominantly been addressed in cells derived from the embryonic neural tube ectoderm (Boissy and Nordlund, 1997). Pigmented cells or melanocytes in the normal adult mammal occur in the skin, uvea, retina, meninges, the inner ear, and the Harderian gland (Boissy, 1998). Such cells are characterized by their ability to synthesize melanin, a process confined within discrete organelles termed melanosomes. Melanosomes share several properties with lysosomes (Orlow, 1995; Raposo et al., 2002), and any melanocyte precursor is defined as a melanoblast (Fitzpatrick et al., 1966). The functions of melanin are not only restricted to absorb, scatter and reflect light, but also include binding of metal ions and organic cations, and acting as antioxidants and scavengers of free reactive radicals (Margalith, 1992; Sarna and Swartz, 1998). Interestingly, increasing information link the functions of the pigmentary and immune systems (Mackintosh, 2001; Raposo et al., 2002).

#### The Melanin Chemical Pathway



#### Thorsen et al.



Pigment Cell Res. 19; 327-336

Biochem Cell Biol. 2012 Dec;90(6):769-78. doi: 10.1139/o2012-033. Epub 2012 Nov 20.

# Melanogenesis in visceral tissues of Salmo salar. A link between immunity and pigment production?

Arciuli M, Fiocco D, Cicero R, Maida I, Zanna PT, Guida G, Horsberg TE, Koppang EO, Gallone A.

### Source

Sezione di Biologia Medica, Dipartimento di Scienze Mediche di Base, Neuroscienze ed Organi di Senso, Università degli Studi di Bari, Policlinico-Piazza Giulio Cesare, 70124 Bari, Italy.



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### Fish & Shellfish Immunology

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# Pigment-producing granulomatous myopathy in Atlantic salmon: A novel inflammatory response

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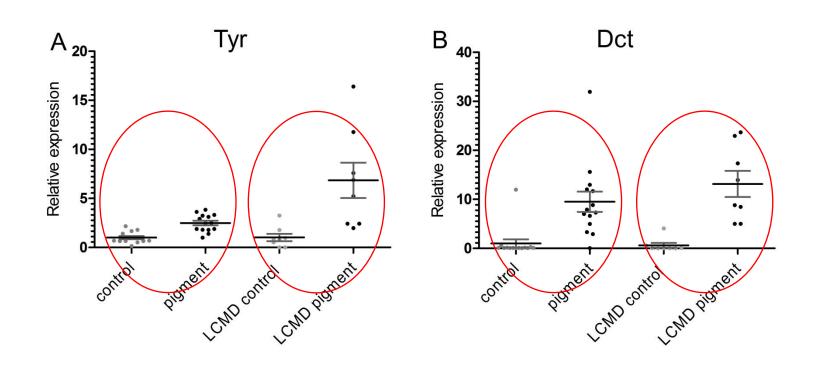
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# Up-regulation of the tyrosinase gene family in the black spots





# The effect of vaccination, ploidy and smolt production regime on pathological melanin depositions in muscle tissue of Atlantic salmon, *Salmo salar* L.

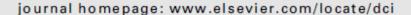
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### Developmental and Comparative Immunology





Transcription of the tyrosinase gene family in an Atlantic salmon leukocyte cell line (SHK-1) is influenced by temperature, but not by virus infection or bacterin stimulation



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### Pathological pigmentation in the hearts of Atlantic salmon (Salmo salar L.) with cardiomyopathy syndrome

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# Er melanin alltid melanin?















8. Kan melanisering unngås?
Ja, tydeligvis!