Tandem mass spectrometry analysis of prostaglandins and isoprostanes

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1

Overview

- **Introduction to prostaglandins (PGs) and their synthesis**
- **Mass spectrometry characterization of PGs and isoprostanes**
- **PGs in Cox-dKO pups and *C. elegans***

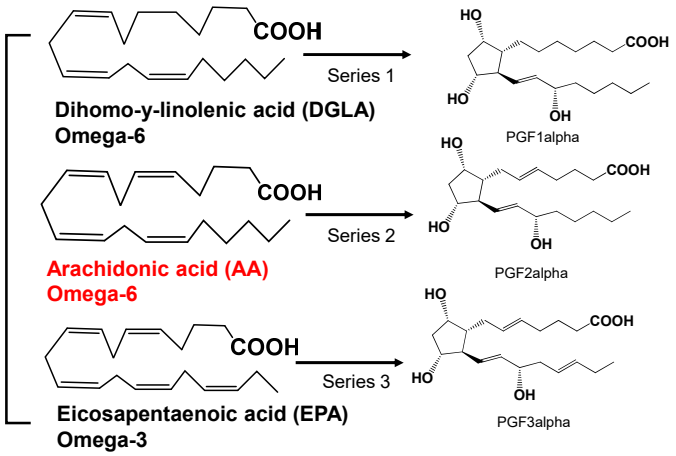
2

Prostaglandins

- Derived from 20 carbon PUFA, have short half-lives and act as local hormones
- Bind to specific cell surface G-protein coupled receptors and implicated in a number of physiological processes including reproductive function.
- NSAIDs acts through inhibiting Cox and hence PGs and exert various effects, including infertility. However, the genetics of prostaglandin synthesis and action have largely been unexplored *in vivo*.
- Mammalian systems are not well suited for discovering new genes and molecular mechanisms involved in PG reproductive functions.
- The nematode *C. elegans* provides a platform for discovering roles of genes and mechanisms that would provide an ideal complement to mammalian systems.

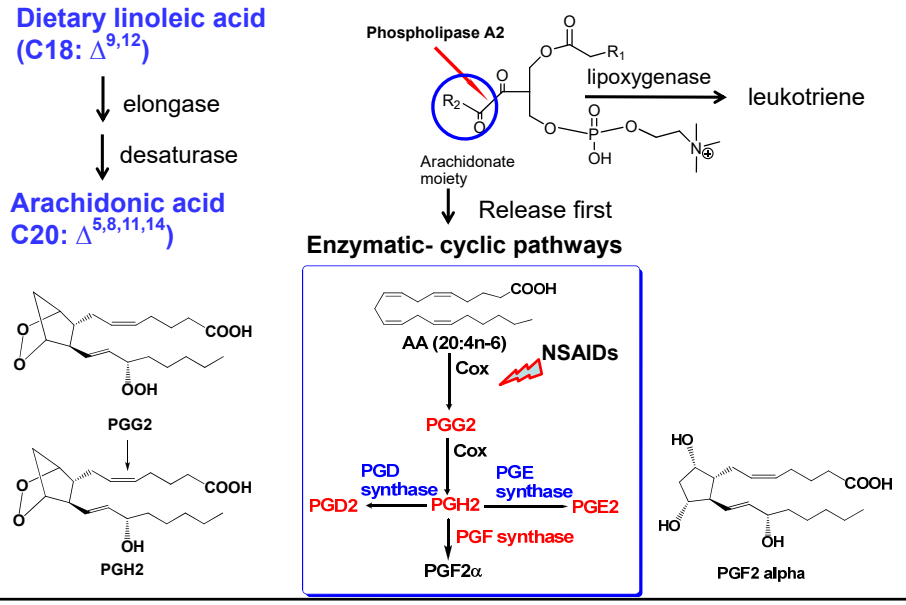
3

Polyunsaturated fatty acids (PUFAs)- substrates for PGs



4

Cox-dependent PGs synthesis



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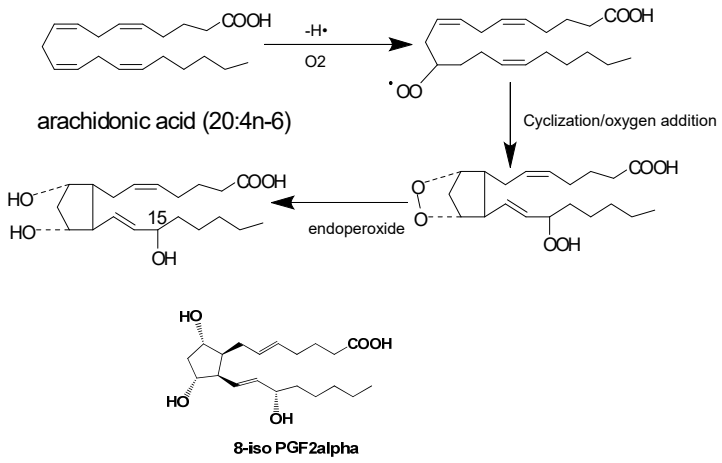
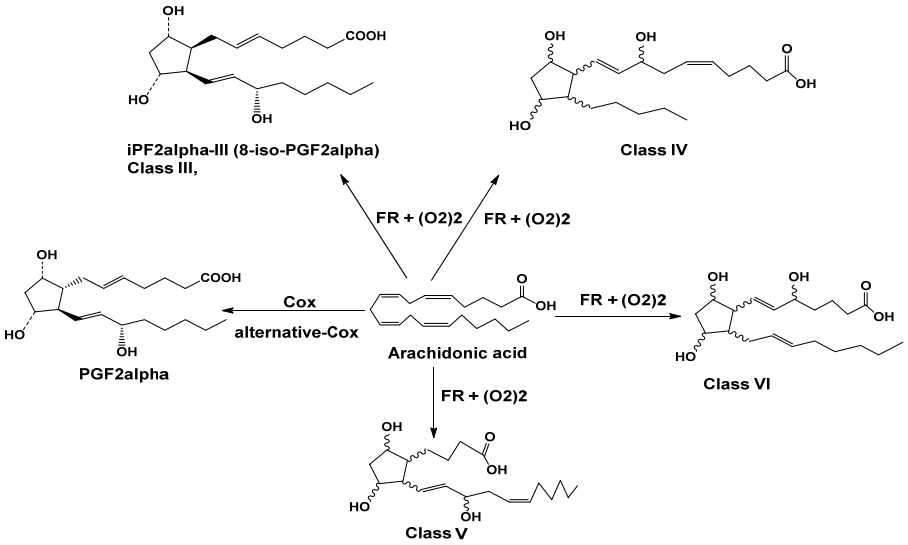
Non-enzymatic F2-isoprostane (F2-IsoP) synthesis



6

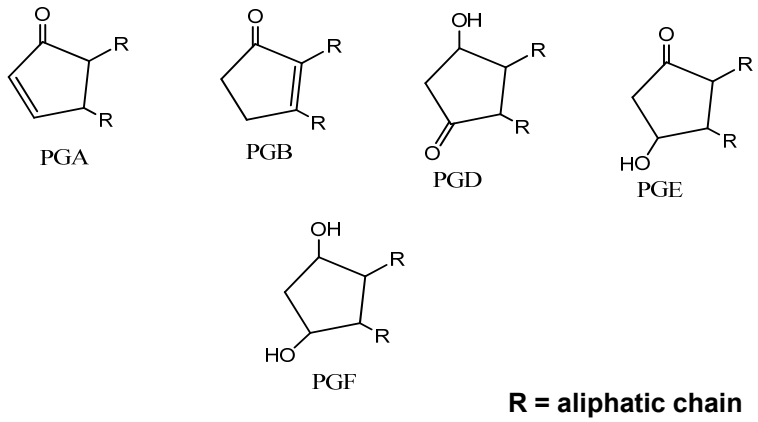
Four classes of F2-isoprostanes from free-radical initiated reaction of arachidonic acid



Adopted from Li et al. PNAS, 1999

7

Structural representation PG based on ring features



R = aliphatic chain

8

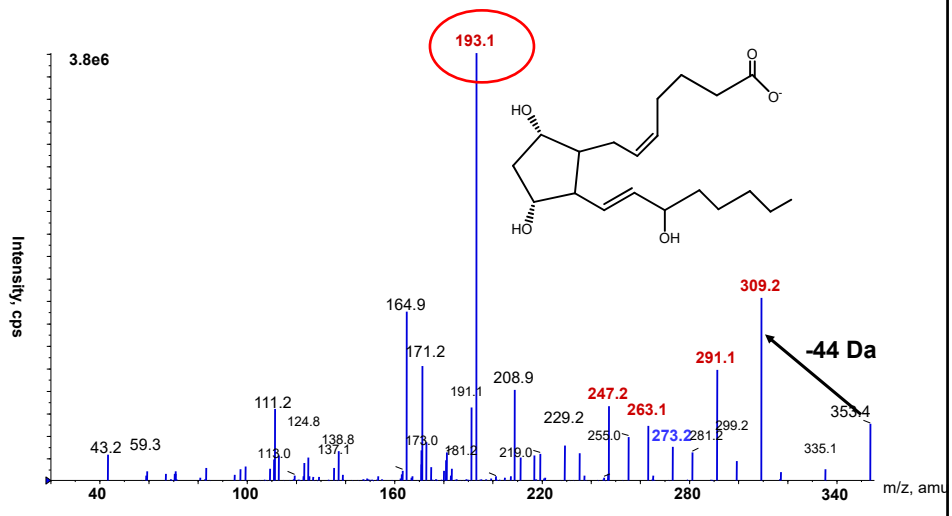
Prostaglandin analysis

Concentration range nM-pM in biological samples

1. **Immunoassay (poor specificity for isomeric PGs, and only one or a few compounds/assay)**
1. **GC-MS (derivatization needed)**
1. **LC-MS/MS**

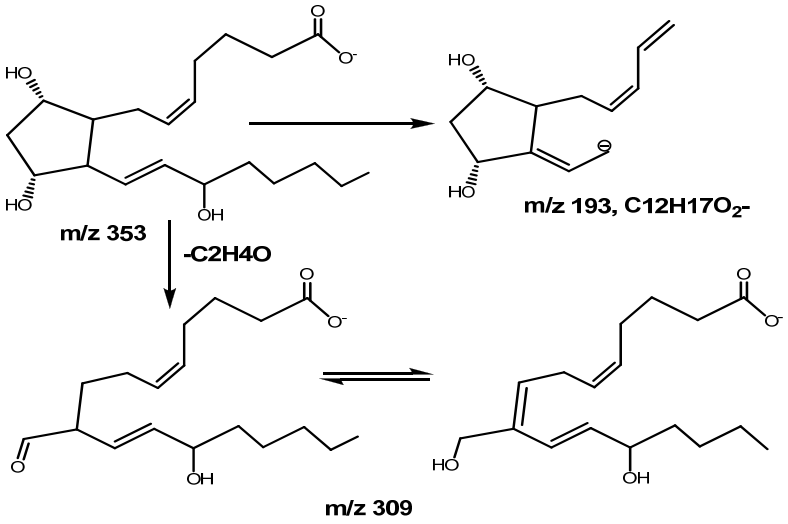
9

ESI-MS/MS of the [M-H]- from PGF2α m/z 353 using a quadrupole mass spectrometer



10

Fragmentation scheme of PGF2α [M-H]⁻ m/z 353

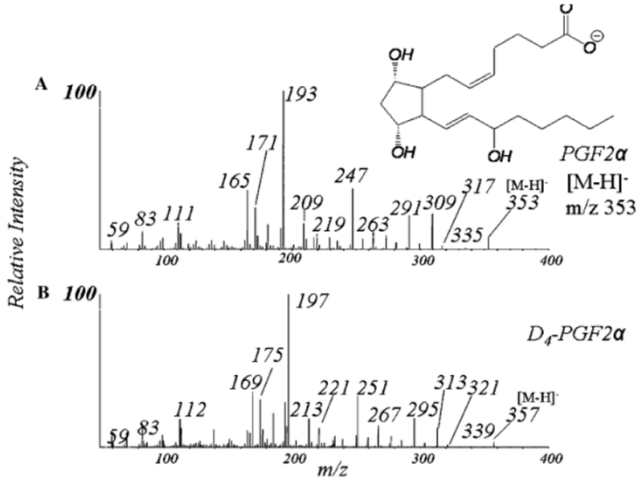


Ions m/z 309, 291, 273 and 193 are indicative of F2-ring

Adopted from Murphy et al. Analytical Biochemistry, 2005

11

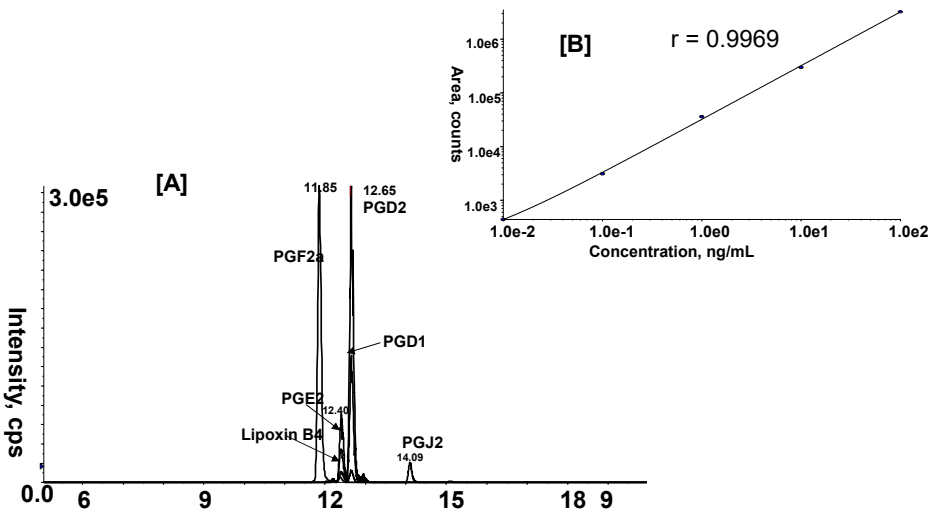
What information does deuterium labeling at C-2 and C-3 of PGF2 provide us for structure elucidation of PG?



Source: Murphy et al. Analytical Biochemistry, 2005

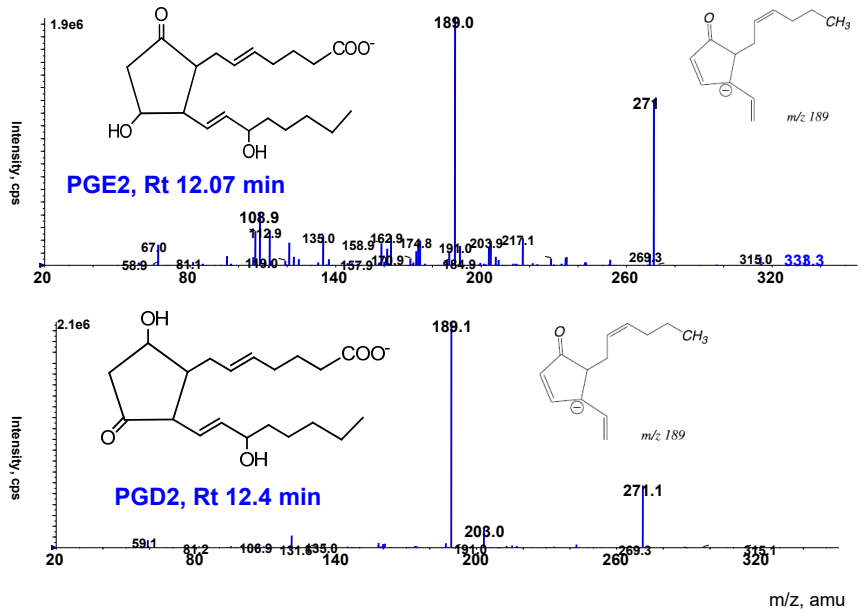
12

Separation of PGs[A] and standard curve of PGF2alpha [B]



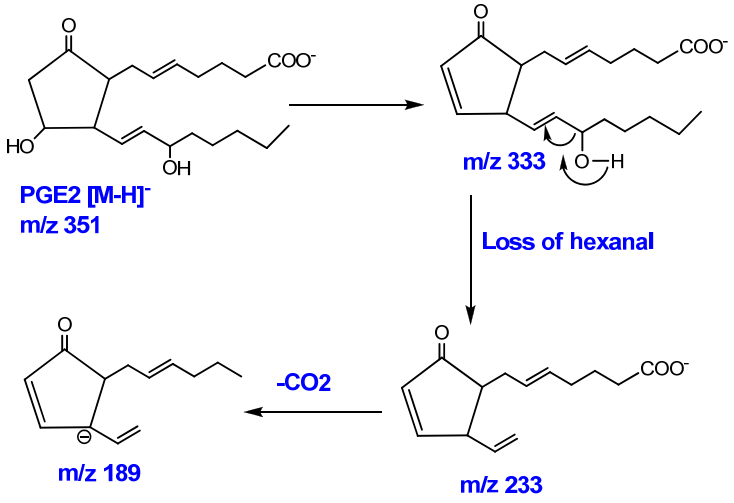
13

MS/MS fragmentation of PGE2 and PGD2 m/z 351.00



14

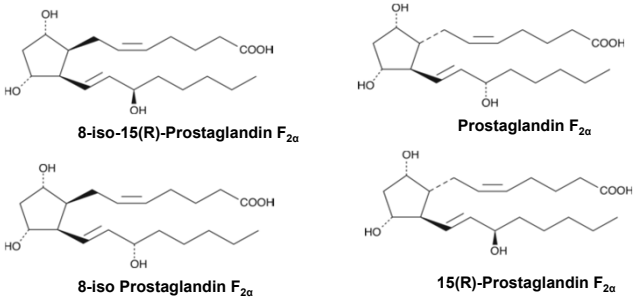
MS/MS fragmentation of PGE_2 $[M-H]^-$ *m/z* 351

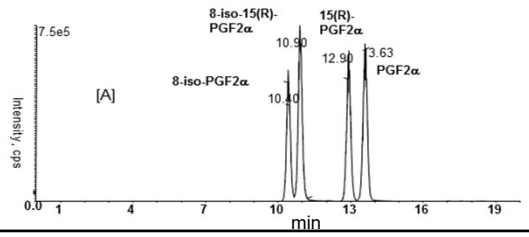


The first loss of water, *m/z* 189 and *m/z* 233 are characteristics of PGE_2/PGD_2

15

PGs and diastereoisomer isoprostanes can be distinguished based on retention time in LC-MS

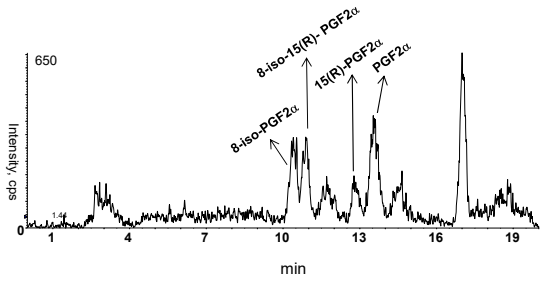




Prasain et al., J Chrom B. 2013

16

SRM chromatogram showing isoprostanes and PG in an AKI patience



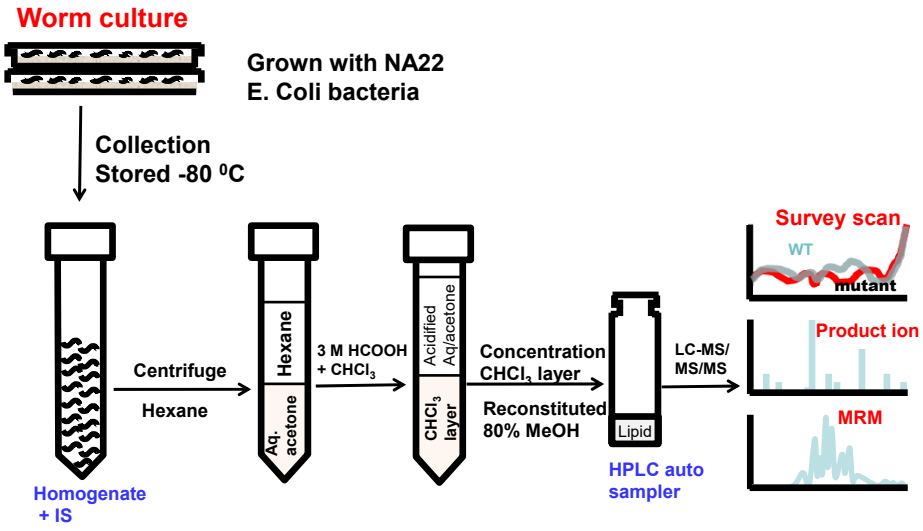
Prasain et al., J Chrom B. 2013

17

Cox-independent PGs

18

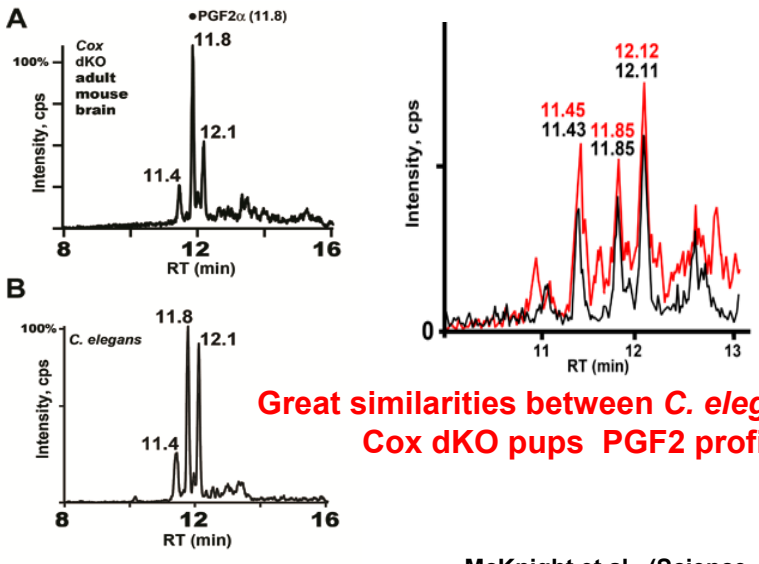
C. elegans culture, lipid extraction and mass spectrometry analysis



Prasain et al., JoVE, 2013

19

Cox-independent PGs is widespread

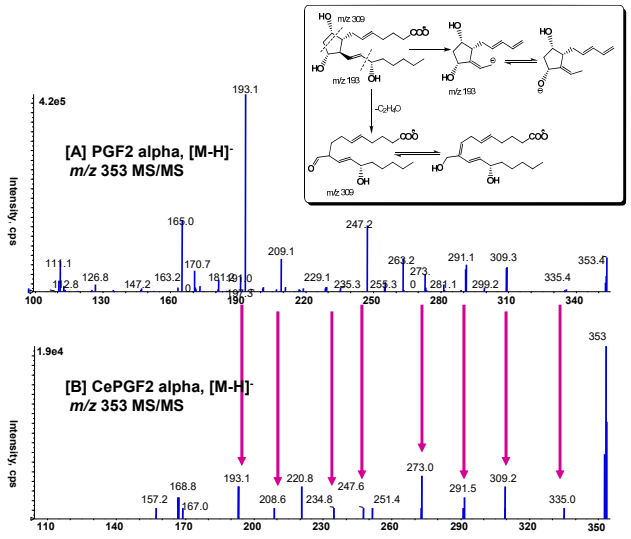


Great similarities between *C. elegans* and Cox dKO pups PGF2 profile

McKnight et al., (Science, 2014)

20

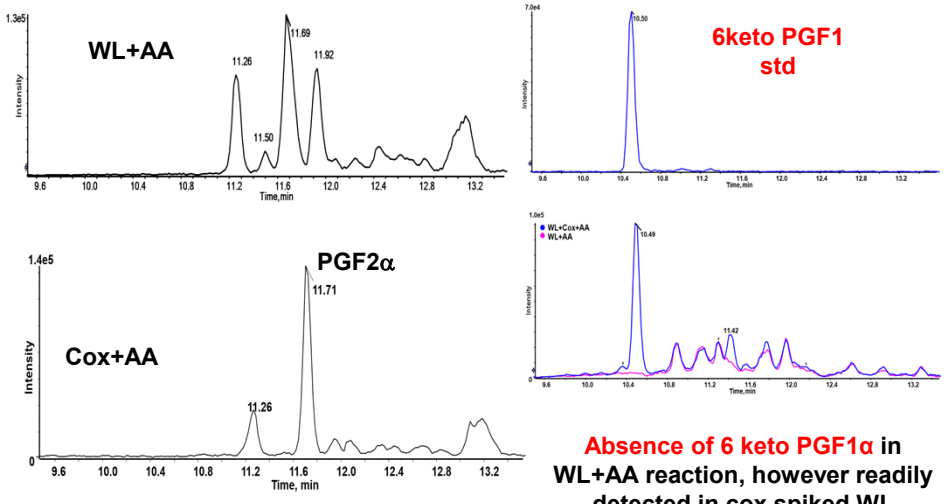
LC-MS/MS of ion *m/z* 353 $[M-H]^-$ from wild type *C. elegans* extract confirmed that $CePGF_2$ is a PGF_2alpha-like PG



Edmonds et al., Dev Cell. 2010

21

Cox-independent F2-PGs with a signature profile, in vitro experiments

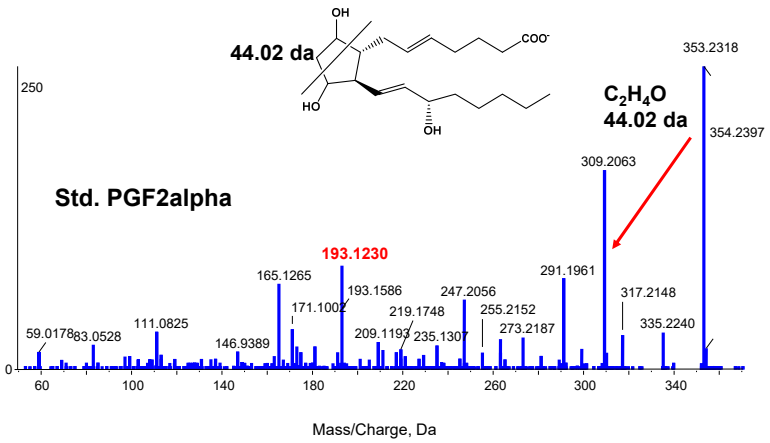


Tiwary et al., Scientific Reports, 2019

Absence of 6 keto PGF1α in WL+AA reaction, however readily detected in cox spiked WL
WL = worm lysate

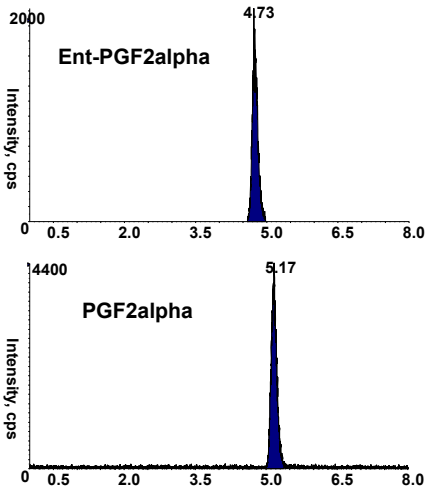
22

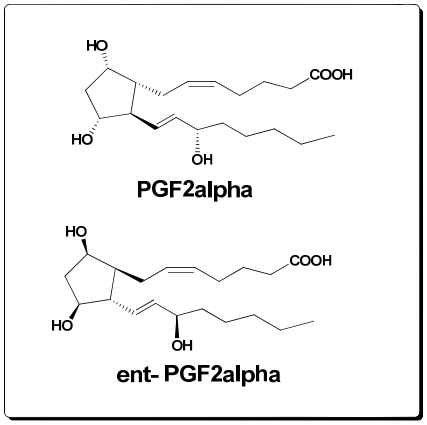
High-resolution mass spectrometry analysis of PGF2alpha



23

Separation of PGF_2alpha and its enantiomer only possible in chiral normal phase column (ChiralPak AD-H column) APCI –ve ion mode

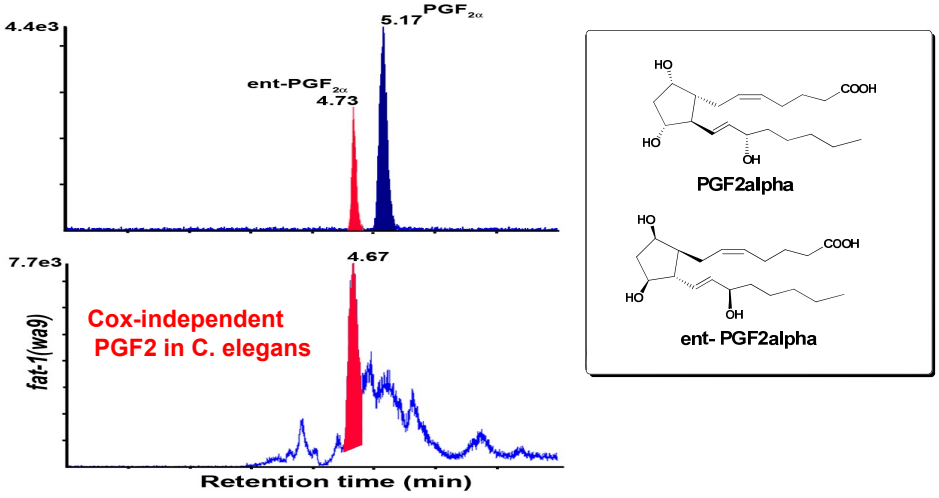




Hoang et al., PLOS Genetics. 2013

24

Cox-independent PGF2 showed close similarity with ent-PGF_{2a} in chiral normal phase LC-MRM



Hoang et al., PLOS Genetics. 2013

25

Conclusions

- **Based on liquid chromatography-tandem mass spectrometry (LC-MS/MS), genetic analyses, and bioactivity assays, *C. elegans* synthesizes Cox-independent F-series PGs from PUFA precursors.**
- **F-series PGs are synthesized in Cox-deficient mice, indicating the possible existence of similar mechanisms in other animals.**

26