Dual-Domain Oxidase 2 (Duox2): A Non-phagocytic NADPH Oxidase Homologue

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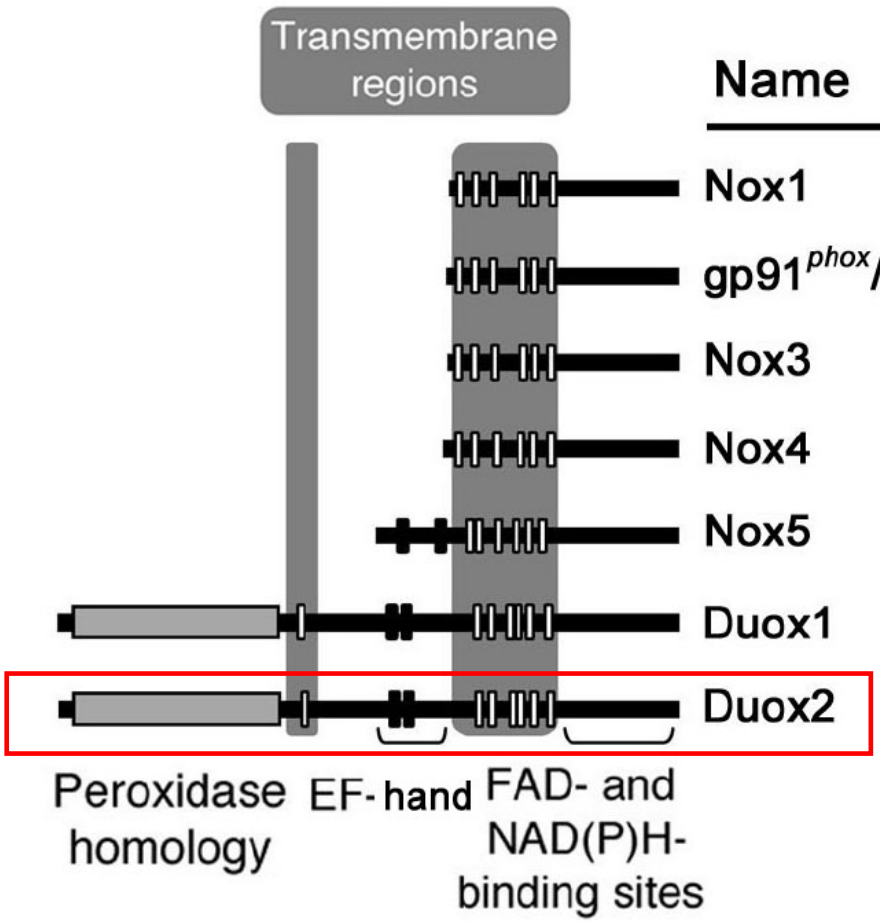
Background

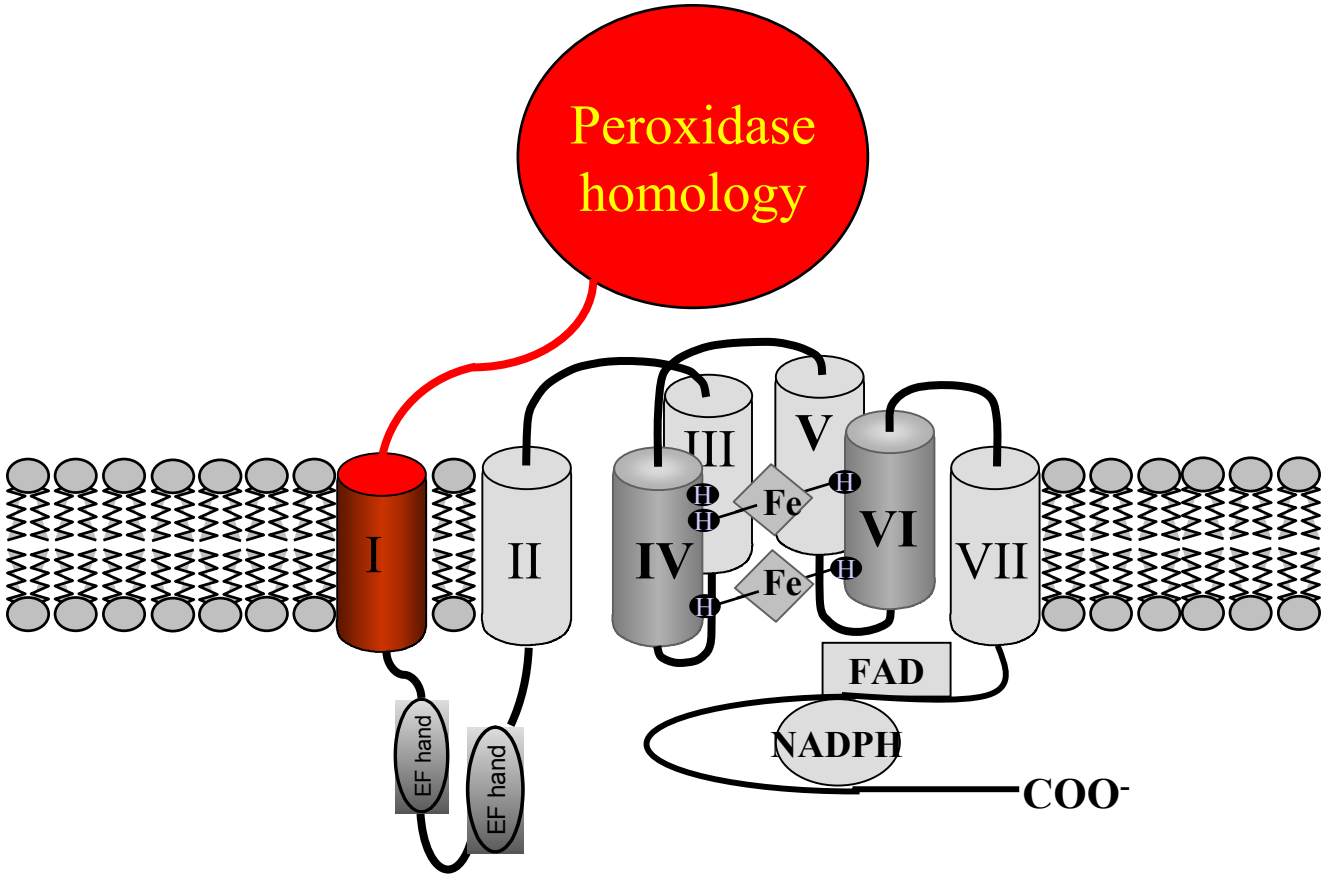
- Phagocytes are vital players in innate immunity, largely due to their ability to generate antimicrobial ROS at the phagocyte membrane.
- The membrane constituent responsible for ROS generation in phagocytes is NADPH oxidase 2 (NOX2, $gp91^{phox}$)
- Recently, non-phagocytic NOX homologues (NOX1, NOX3, NOX4, NOX5, Duox1, Duox2) have been identified.
- These NOX homologues use electrons from NADPH to generate superoxide, which dismutes to form H_2O_2.
- However, the biological importance of the NOX proteins is poorly understood.
- This presentation will discuss the NOX protein Dual-domain Oxidase 2 (Duox2).

The NADPH Oxidase (NOX) Family



Name	Expression
Nox1	Colon epithelium
gp91^{phox}/Nox2	Phagocytes, B cells
Nox3	Inner ear
Nox4	Kidney, osteoclasts, vasculature
Nox5	Testis, lymphoid organs
Duox1	Thyroid, airway epithelium
Duox2	Thyroid, GI, salivary glands, mammary glands, airway epithelium

Structure of Duox2



- 7 putative transmembrane domains
- 4 NADPH-binding domains
- 1 FAD-binding domain
- 2 EF hand motifs (predicted to bind Ca^{2+})
- Peroxidase domain

Genomic Characterization

- The Duox2 gene is found on chromosome 15q15, arranged in a head to head configuration with Duox1 (another NOX homologue)
- Contains 34 exons spanning 21.5 kb

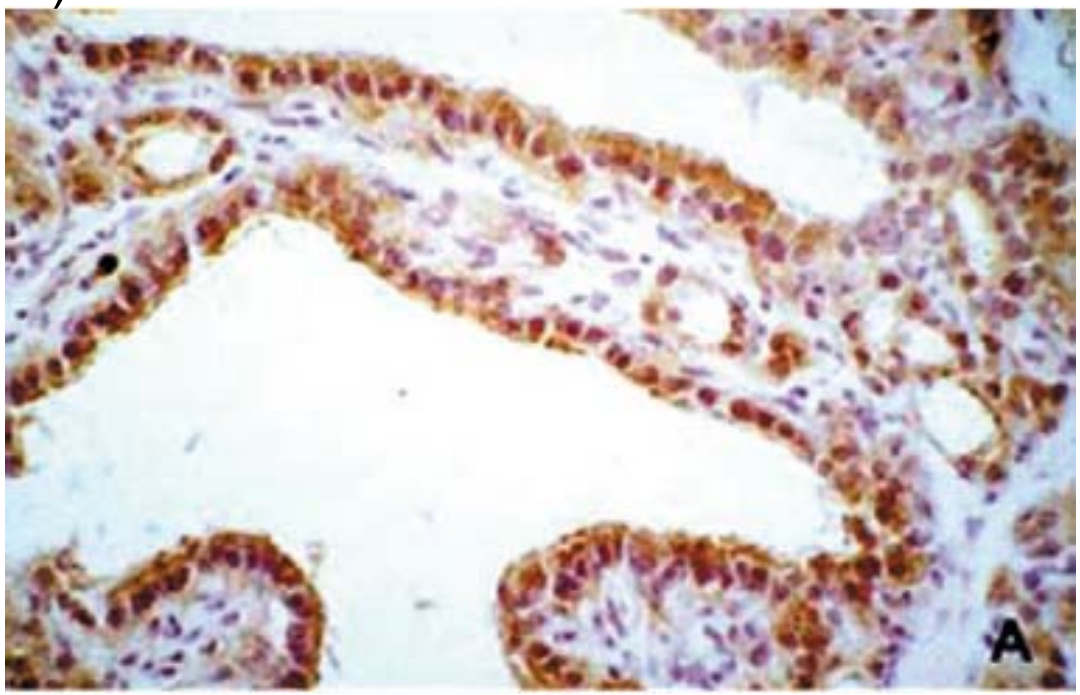
Schematic of genomic organization. Boxes represent exons. Adapted from [7].

Message

- mRNA is 6126 nt
- Expressed predominantly in the thyroid, gastrointestinal epithelium, respiratory epithelium, mammary glands, salivary glands, and prostate.
- Expression only characterized in thyroid tissues, in which cAMP induced upregulation

Protein

- 1548 aa
- 2 glycosylation states: (i) fully mature glycosylated form (190 kDa) expressed at apical membrane, and (ii) the high mannose glycosylated immature form expressed exclusively within the ER (180 kDa)
- In thyroid tissues, Duox2 is localized to the apical border of thyrocytes (see below)



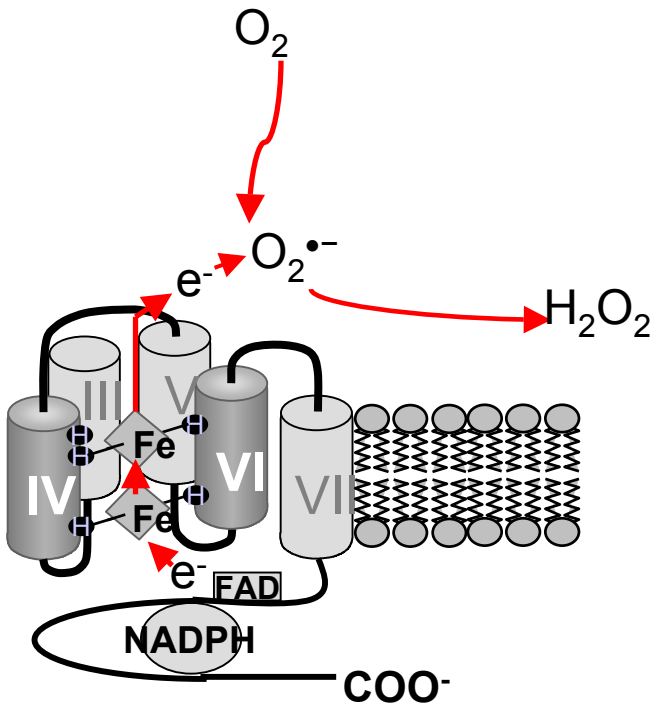
Duox2 is expressed at the apical membranes of thyrocytes

Duox2 Biochemistry

- Thyrocyte membrane particulates have been known to possess an NADPH-dependent H_2O_2-generating system [2], whose activity required Ca^{2+} in micromolar concentrations [3].
- Ca^{2+} suggested to induce a conformational change, allowing NADPH access to oxidase active site [4].
- Based on functional similarities of H_2O_2 generation in the thyroid and phagocyte system, this machinery was determined to be Duox2 (& Duox1).

Mechanism of H_2O_2 Production

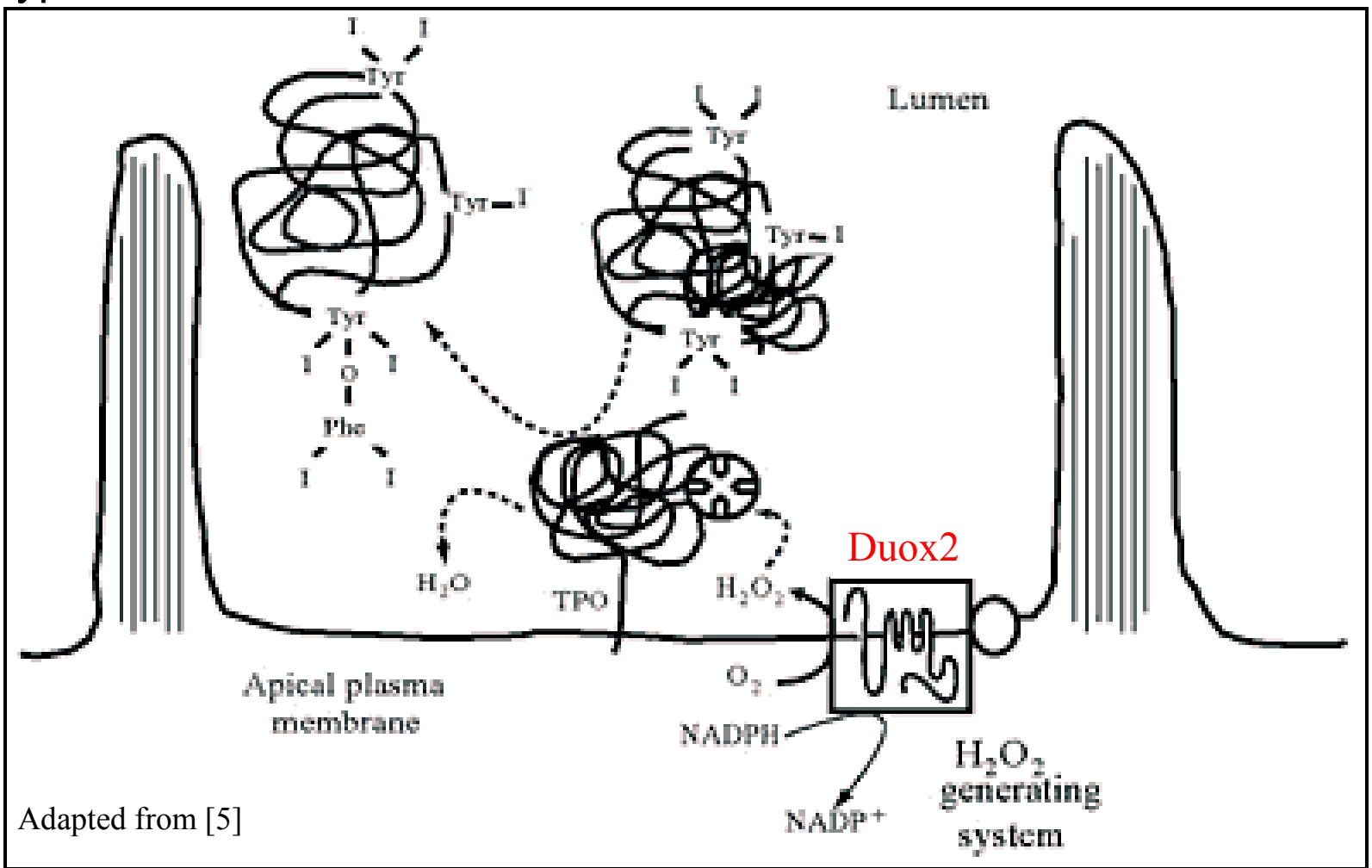
- The mechanism of H_2O_2 production is poorly characterized, but is predicted to proceed through superoxide.
- The NOX system (e.g., Nox2) catalyzes $1e^-$ transfers from NADPH → FAD → heme group → molecular O_2, generating superoxide ($O_2^{\bullet-}$).
- Duox2 is thought to hold this superoxide intermediate within the active site until another e^- is transferred, thus generating H_2O_2.



Duox2 Role *in vivo*

• The presence of H_2O_2 at the apical border of thyrocytes has been known for years to be required for thyroid hormone synthesis.

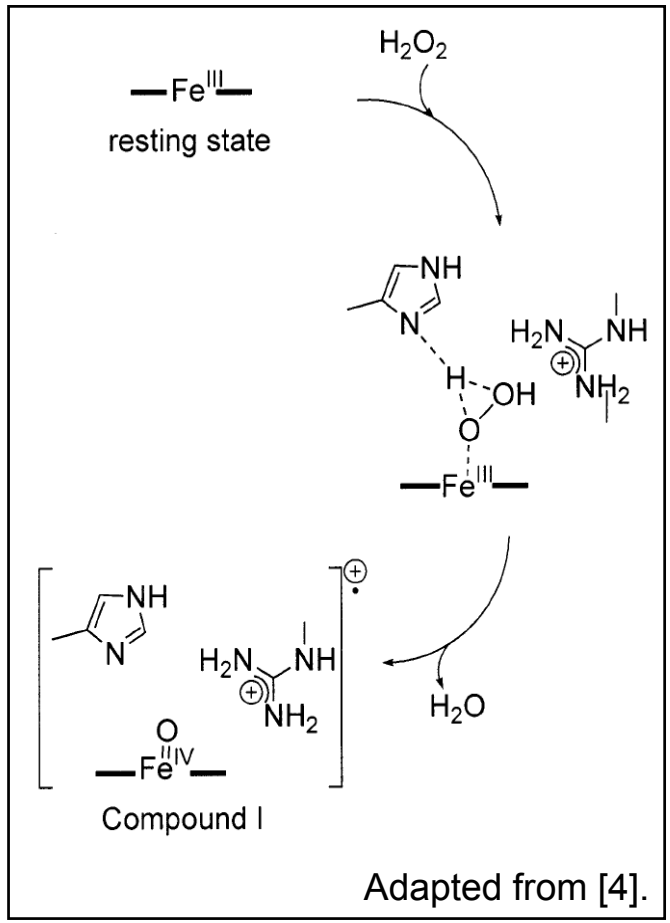
•However, the source of the H_2O_2 has remained elusive. It is hypothesized that Duox2 serves this function.



Adapted from [5]

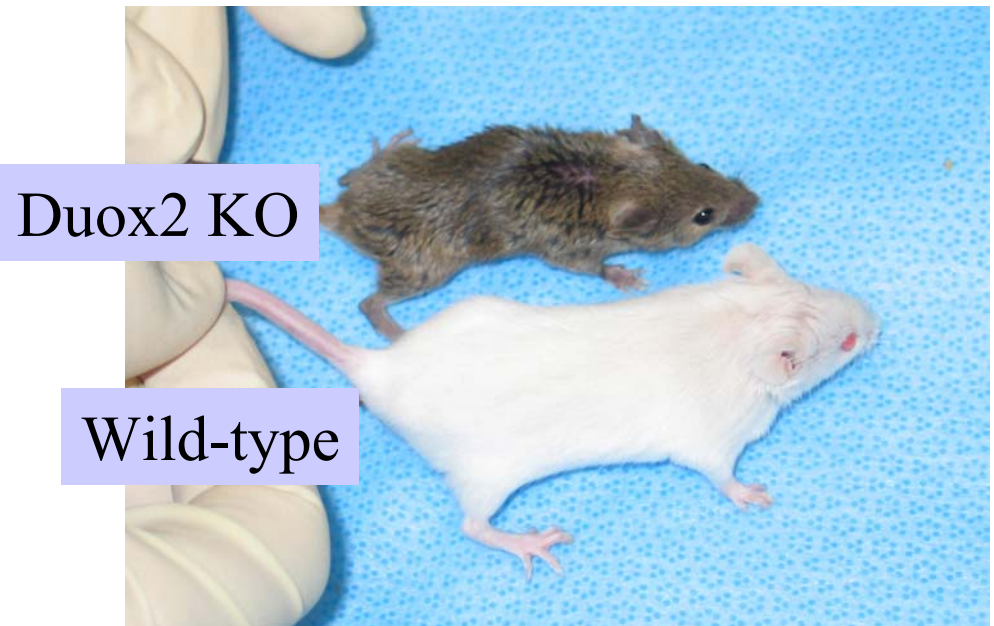
Role of H_2O_2 in thyroid hormone synthesis

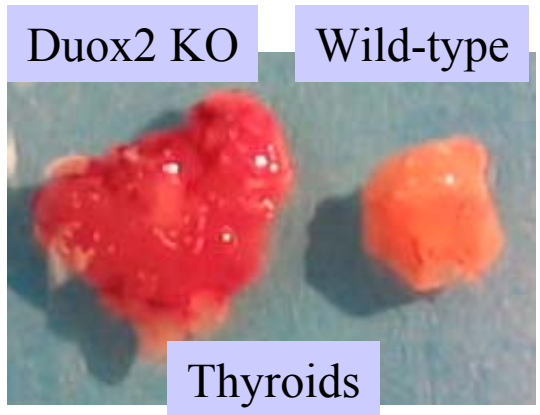
1. H_2O_2 reacts with TPO to form Compound I, in which one of the oxidizing equivalents of H_2O_2 is stored on the iron as an Fe(IV) oxyferryl moiety and the other on the porphyrin ring as a π radical cation. The π radical cation can be transferred to a protein side chain (see figure).
2. Compound I oxidizes iodide (I^-) to form iodine (I^0), which can react with tyrosyl residues of thyroglobulin forming iodotyrosines.
3. H2O2 is also necessary for the coupling of iodotyrosyl residues to form thyroid hormones.



Evidence That Duox2 is Involved in Thyroid Hormone Synthesis

- Duox2 knockout mice support the role for Duox2 in thyroid hormone synthesis.
- Mice deficient in Duox2 exhibit decreased growth (left figure) and goiterous thyroids (right figure). Both are highly suggestive of hypothyroidism
- Duox2-deficient mice exhibited markedly lower levels of thyroid hormones (free thyroxine (T_4) and total T_4) [6].





Summary

- Duox2, a NADPH oxidase homologue, is required for thyroid hormone synthesis.
- Its activity requires both Ca^{2+} and NADPH.
- The production of H_2O_2 is hypothesized to proceed through a superoxide intermediate.
- Its role in non-thyroid tissues remains to be determined.

References

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3. Deme D, Pommier J. (1985) NADPH-dependent generation of H2O2 in a thyroid particulate fraction requires Ca2+. *FEBS Lett.* **186(1):** 107-110.
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