# Trojan, a possible regulator of apoptosis, belongs to a novel protein family

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## Introduction

Aiming to identify novel proteins related to the immune system, we cloned a previously unknown antigen from chicken (*Gallus gallus*). The molecule is a leukocyte-specific, cell surface protein that we named "Trojan".

Trojan belongs to a novel gene family, that in chicken includes two more members, called "Mystran" and "Thracian". Here, we characterise Trojan, investigate its function and perform evolutionary analyses of its family.

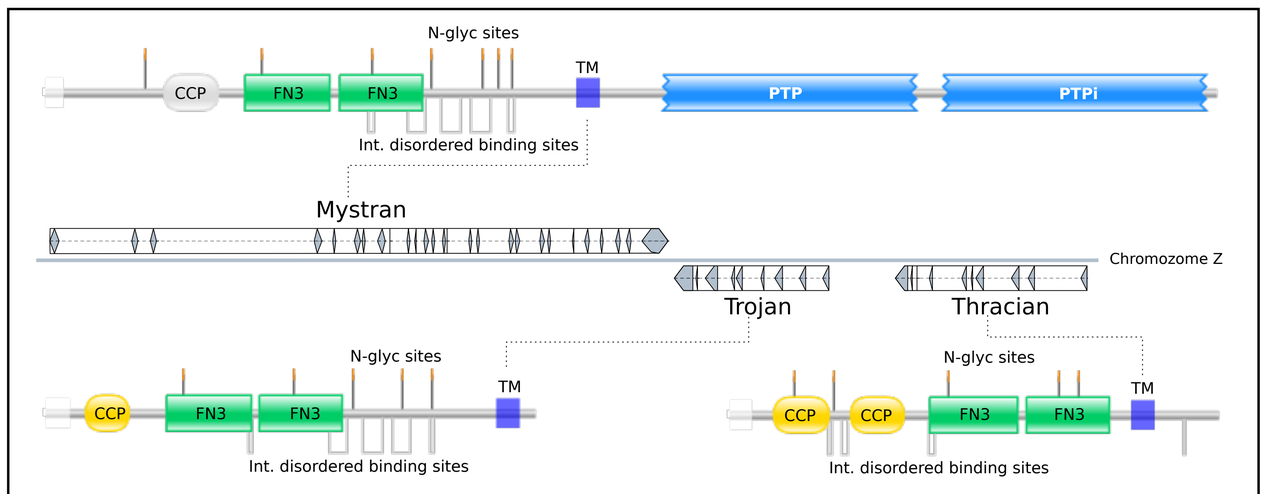
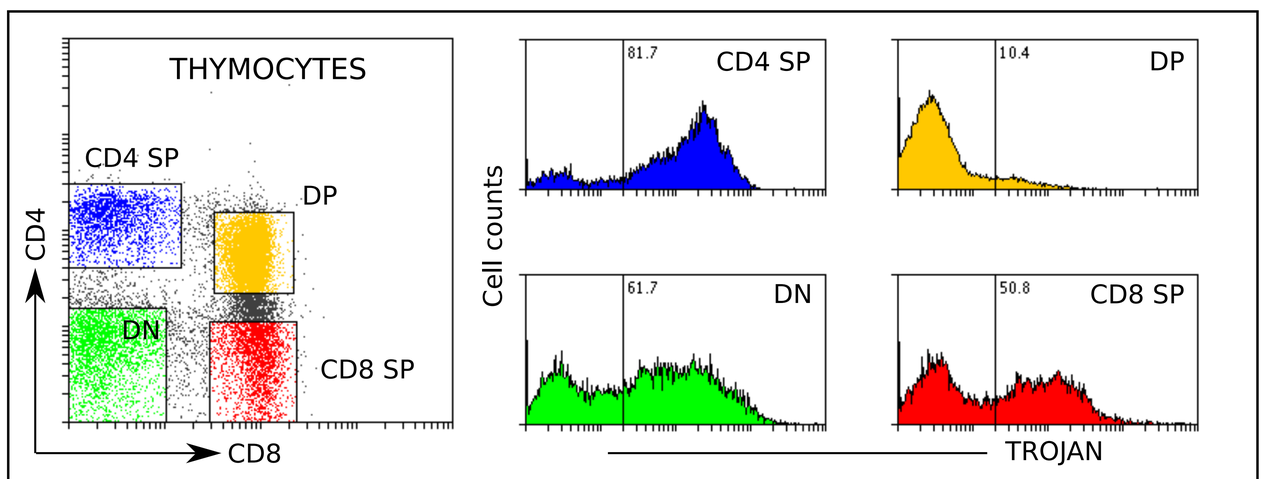



**Figure 1. Trojan family.** The three genes reside on chromozome Z. Mystran is predicted as a receptor type protein tyrosine phosphatase and Thracian -- as a transmembrane protein having an ITAM.



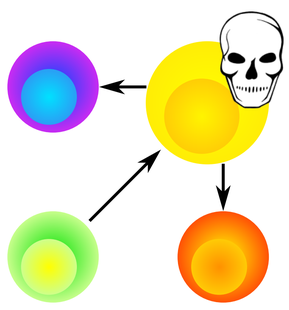



**Figure 2. Trojan diminishes from the surface of double positive thymocytes.** Similar expression pattern is observed for the anti-apoptotic BCL-2 and the pro-survival IL-7R. In thymus, cells at the double positive stage are subject to selection processes and the majority die by apoptosis. Therefore, we hypothesise an anti-apoptotic or proliferative role for Trojan.

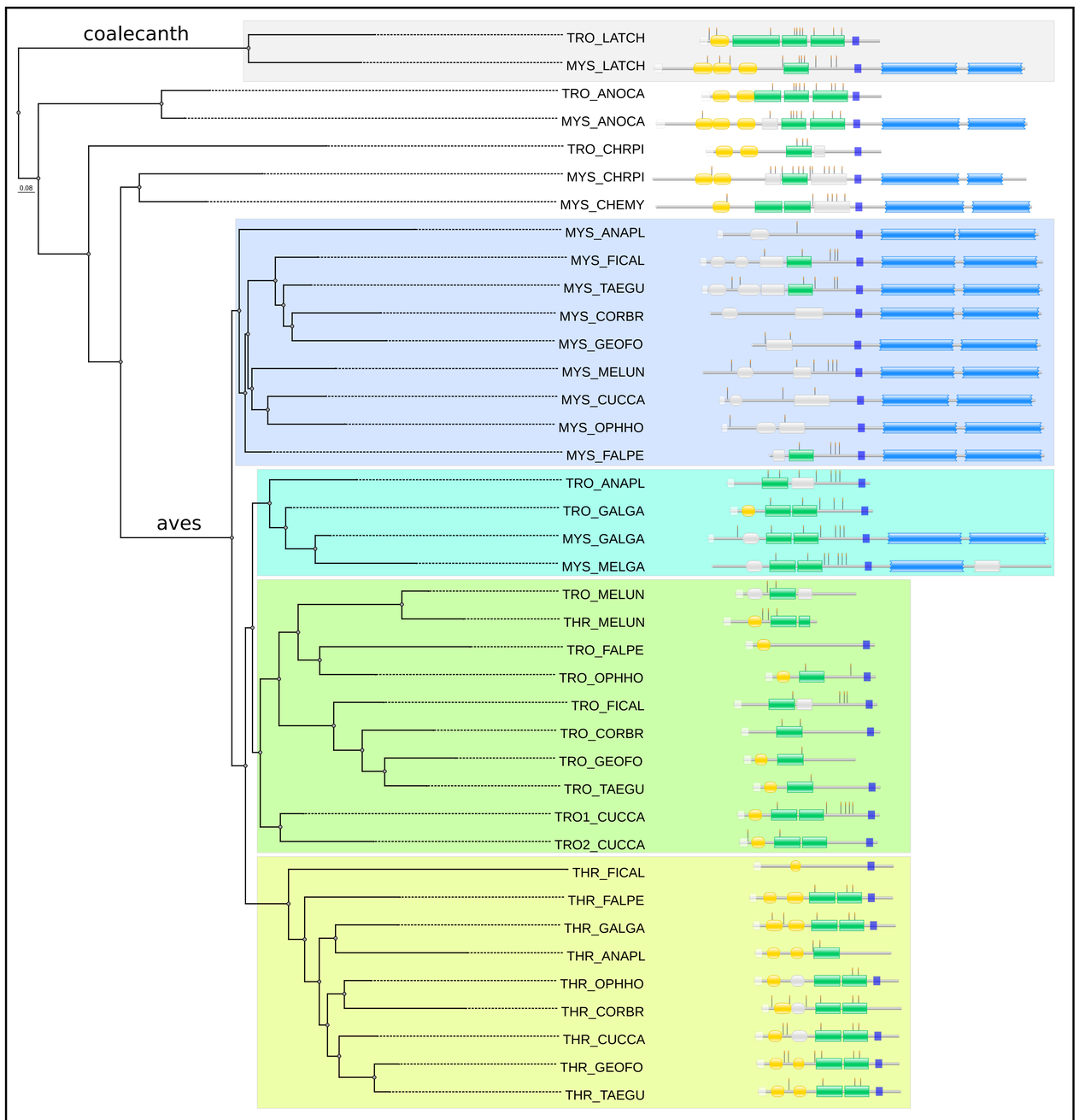



**Figure 5. Trojan family exists in other avian species, reptiles and coalecanth fish.** Mystrans, Trojans and Thracians form three major clusters. The orthologues do not exactly recapitulate the species tree, likely due to gene conversions between the family members.

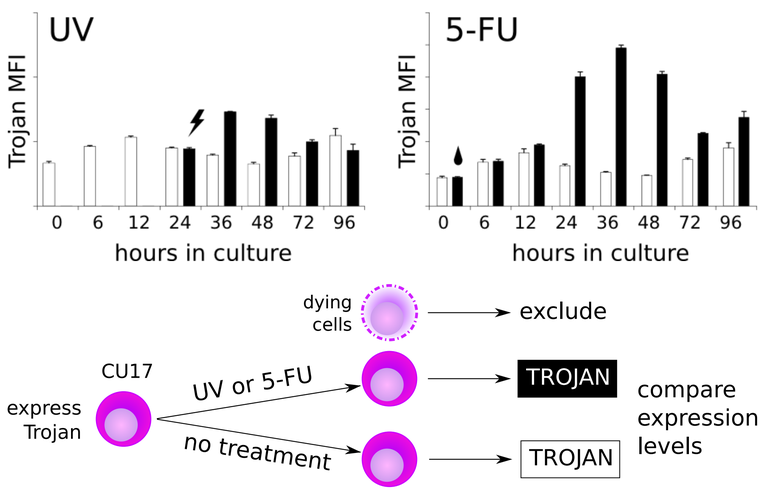



**Figure 3. Upon apoptosis induction, Trojan expression increases on surviving cells.** Chicken T cell line CU17 was treated with ultraviolet light or 5-FluoroUracil. Trojan expression rises on the surface of non-apoptotic cells (black bars), as compared to non-treated cells (white bars).

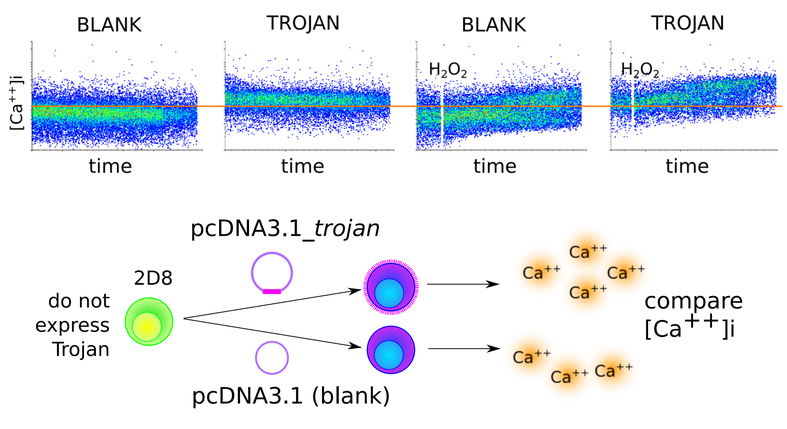



**Figure 4. Trojan over-expressing cells show elevated $[Ca^{++}]_i$.** Cells (2D8) were transfected with expression vector and later loaded with calcium indicators. Cells expressing Trojan have higher calcium baseline levels, as compared to mock transfected cells.

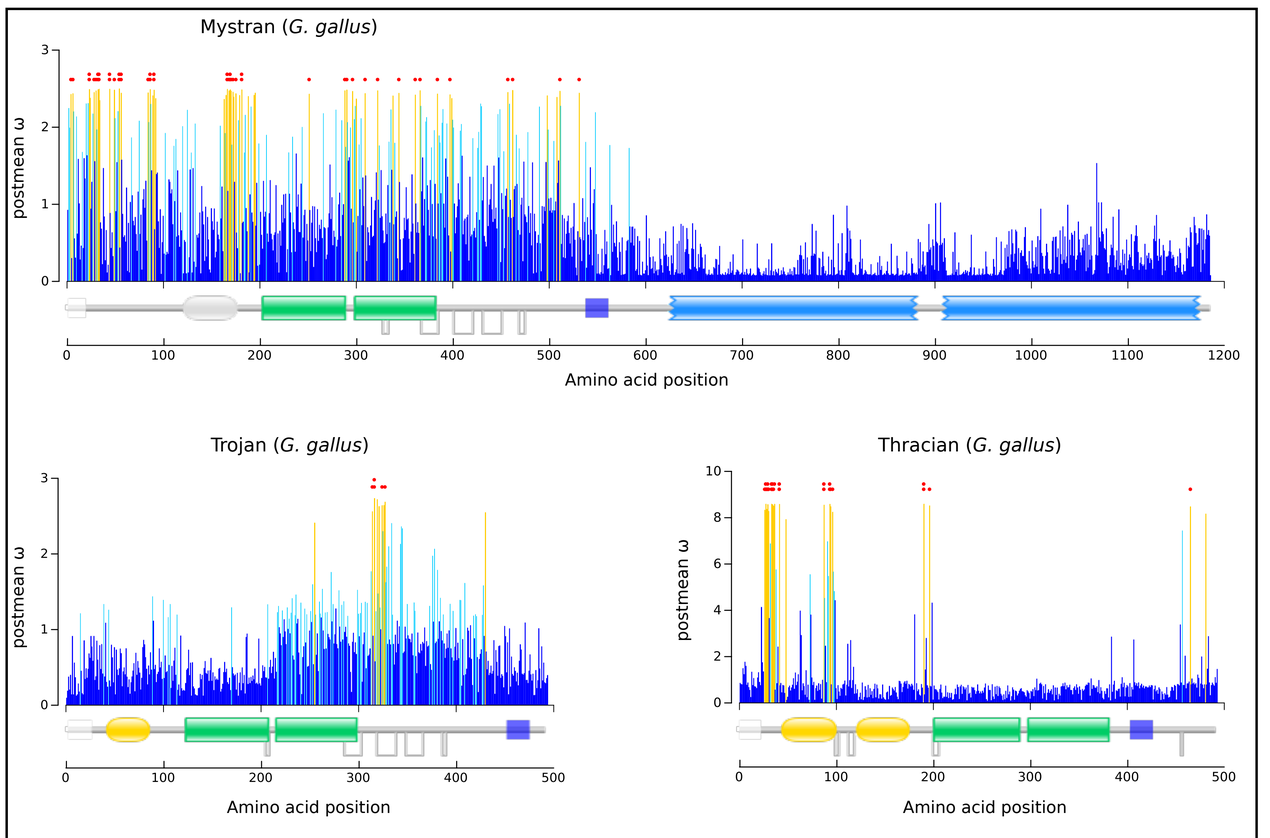



**Figure 6. Evolutionary selection of Mystran, Trojan and Thracian.** Selected sites with probability greater than 90% are shown in orange, and sites with probability higher than 95% and 99% are indicated by one or two dots, respectively.

Petrov P., Motobu M., Salmi J., Uchida T., Vainio O., 2010 **Novel leukocyte protein, Trojan, differentially expressed during thymocyte development. J Mol. Immunol.** 47, 1522-1528

Petrov P. , Syrjänen S., Smith J., Gutowska M.W., Uchida T., Vainio O., Burt D.W., 2015 **Characterization of the avian Trojan gene family reveals contrasting evolutionary constraints.** PloS ONE, accepted