Cellular Genetics

across metazoans by analysis of synteny and rare genomic characters

GK635

Abhishek Kumar and Hermann Ragg

Department of Biotechnology, Faculty of Technology, University of Bielefeld, D-33501 Bielefeld, Germany, Email: hr@zellkult.techfak.uni-bielefeld.de

Introduction

The serpins represent a superfamily of proteins with a common fold (Figure 1a) that cover an extraordinary broad spectrum of different biological functions. Most serpins inhibit proteases from one or several different clans of peptidases (inhibitory serpins); some superfamily members, however, exert disparate roles, such as assisting in protein folding or transportation of hormones (non-inhibitory serpins). This functional diversity is enabled, at least in part, by the unusual structural plasticity of the serpin molecule that, in the native form, often takes a metastable structure. Serpins can perform their activity in the extracellular space or in various subcellular compartments, including the secretory pathway routes and they are found in all high-order branches of the tree of life. Deficiency of some serpins, such as antithrombin or neuroserpin, is lethal or may be associated with serious pathology. Mutations of the neuroserpin gene for instance may result in formation of intracellular aggregates in the brain causing dementia, while wild type neuroserpin provides protection of neuronal cells in cerebral ischemia and other pathologies. Vertebrate serpins are classified into six groups based on gene structures with neuroserpin belonging to group 3 (Figure 1b). However, the evolutionary roots of serpins beyond the fish/tetrapod split are unresolved.

Here we illustrate how rare genomic characters and analysis of synteny helped in elucidating the phylogeny of secretory pathway serpins across metazoa.

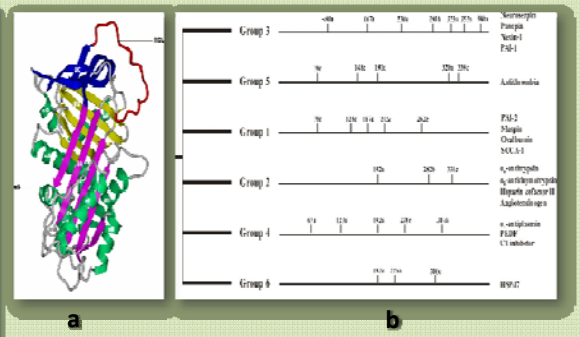

Figure 1 – Structure of a typical serpin (a) and gene structure-based phylogenetic classification of vertebrate serpins (b).

Methods

Mining of serpin DNA and protein sequences and microsynteny analysis

Serpin protein and DNA sequences of various genomes were extracted from publicly accessible databases via the BLAST software package (including PSI-BLAST) using key words or the human α1-antitrypsin sequence for searching. Chromosomal microsynteny analysis was performed using the NCBI Map Viewer, the ENSEMBL genome browser, the JGI genome browser, the *Tetraodon* genome browser, the UCSC genome browser and inspecting the *Strongylocentrotus purpuratus* genome database.

Sequence alignments, gene structure analyses and mapping of intron positions

Alignments of protein sequences were performed with CLUSTAL X and refined manually in GeneDoc. Intron positions were identified and assigned with GENEWISE. Mature human α1-antitrypsin was used as reference for mapping of positions and phasing of introns in serpin genes.

Results

- Rare genomic characters show that orthologs of neuroserpin exist in early diverging deuterostomia and probably also in cnidarians, indicating that the origin of a mammalian serpin can be traced back far in the history of eumetazoans (Figure 2-5).
- The neuroserpin gene and the *PDCD10 (programmed cell death 10)* gene are found conserved in a head-to-head orientation from vertebrates to sea urchins. (Figure 2).
- Massive changes in the exon-intron organisation of serpin genes have occurred along the lineage leading to vertebrate neuroserpin. (Figure 3).
- A C-terminal address code assigning association with secretory pathway organelles is present in all neuroserpin orthologs, suggesting that supervision of cellular export/import routes by antiproteolytic serpins is an ancient trait, though subtle functional and compartmental specializations have developed during their evolution (Figure 4).
- The *Nematostella vectensis* serpin gene Nve-Spn-1 may represent the earliest diverging neuroserpin ortholog, though orthology with the deuterostome counterparts is currently only supported by protein-based signature sequences (Figures 4-5).

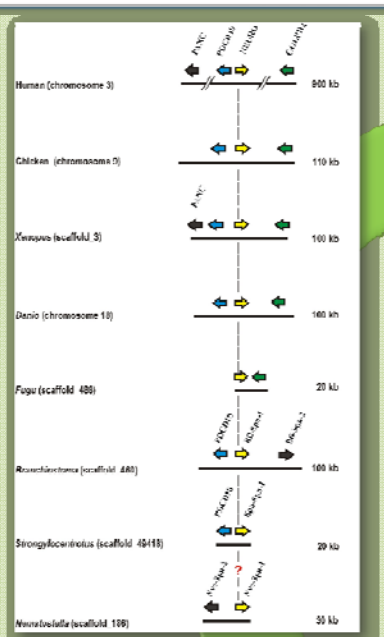

Figure 2 – Micro-synteny of the genes coding for neuroserpin homologs and flanking genes in metazoans.

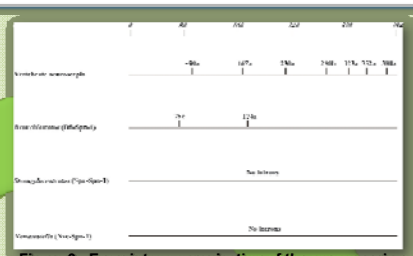

Figure 3 - Exon-intron organisation of the neuroserpin gene lineage. Numbering according to human α1-antitrypsin sequence.

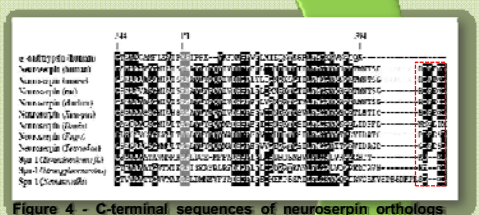

Figure 4 - C-terminal sequences of neuroserpin orthologs from Deuterostomes and serpin Spn-1 from *Nematostella vectensis*.

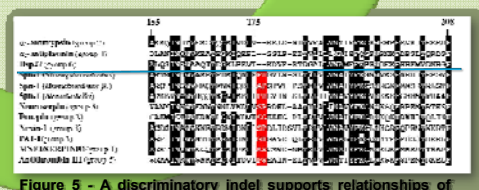

Figure 5 - A discriminatory indel supports relationships of neuroserpin and homologs from sea urchins, lancelets, and *Nematostella*.

Conclusions

- Analysis of microsynteny and other rare characters can provide insight into the intricate family history of metazoan serpins.
- Serpins with the capacity to defend the main cellular export/import routes against uncontrolled endogenous and/or foreign proteolytic activity represent an ancient trait in eukaryotes that has been maintained continuously in metazoans though subtle changes affecting function and subcellular location have evolved.
- The intron distribution pattern of neuroserpin gene orthologs has undergone substantial rearrangements during metazoan evolution.

References

1. Kumar, A. & Ragg, H. (2008). Ancestry and evolution of a secretory pathway serpin. BMC Evolutionary Biology, *8:250.*
2. Ragg, H., *et al.* (2001) Vertebrate serpins: Construction of a conflict-free phylogeny by combining exon-intron and diagnostic site analyses. *Mol Biol Evol*, **18**, 577-584.
3. Bentele C., *et al.* (2006) A proprotein convertase-inhibiting serpin with an ER targeting signal from *Branchiostoma lanceolatum*, a close relative of vertebrates. *Biochem J*, **395,** 449-456.

Acknowledgements

This work was supported by the Deutsche Forschungsgemeinschaft, Graduate Program 'Bioinformatics' at the University of Bielefeld.