

GYF Domains

A Class of Proline-Rich Ligand Binding Adaptor Domains

Dissertation

zur Erlangung des akademischen Grades des
Doktors der Naturwissenschaften (Dr. rer. nat.)

eingereicht im Fachbereich Biologie, Chemie, Pharmazie
der Freien Universität Berlin

vorgelegt von
Michael Kofler
aus Isny

Februar 2006

1. Gutachter: Dr. Christian Freund
Protein Engeneering Group
Freie Universität Berlin und
Leibniz-Institut für Molekulare Pharmakologie (FMP)
Robert-Rössle-Str. 10
13125 Berlin
Deutschland

2. Gutachter: Prof. Dr. Hartmut Oschkinat
Freie Universität Berlin und
Leibniz-Institut für Molekulare Pharmakologie (FMP)
Robert-Rössle-Str. 10
13125 Berlin
Deutschland

Weitere Kommissionsmitglieder:

Prof. Dr. Christine Lang, Prof. Dr. Ferdinand Hucho und Dr. Ludwig Krabben

Datum der Disputation: 28.06.2006

*La lutte elle-même vers les sommets suffit à remplir un cœur d'homme;
il faut imaginer Sisyphe heureux.*

- Albert Camus -

meiner Mutter

Contents

Danksagung	XI
Publications	XIII
Zusammenfassung	XV
Summary	XVI
Abbreviations, Symbols, and Units	XVII
1 Introduction	1
1.1 Adaptor Domains	2
1.1.1 Binding Properties of Adaptor Domains.....	2
1.1.2 Functional and Evolutionary Consequences of the Binding Properties	4
1.1.3 Examples for Adaptor Domain Networks.....	5
1.2 Special Features of Proline	5
1.3 Proline-Rich Sequence Recognition Domains.....	7
1.3.1 Association of Proline-Rich Sequence Recognition Domains with Human Diseases	7
1.3.2 Binding Modes of Proline-Rich Sequence Recognition Domains	7
Recognition of the Pointed End of PPII Helices—EVH1 Domains	10
Recognition of xP Dipeptides—SH3, WW, GYF, and UEV Domains	11
1.4 Sequence Alignment of GYF Domains.....	12
1.5 Structure of GYF Domains	14
1.5.1 Structure of CD2BP2-GYF	14
1.5.2 Structure of CD2BP2-GYF in Complex with the CD2 Peptide.....	16
1.5.3 Structure of SMY2-type GYF Domains	17
1.5.4 GYF Domain Related Folds.....	17
1.6 Functional Context of GYF Domains	19
1.6.1 Involvement of CD2BP2 in T cell Signaling.....	19
1.6.2 Spliceosomal Functions of GYF Domains	20
Mechanism of Splicing	20
Assembly and Composition of the Spliceosome	21
Involvement of GYF Domain Containing Proteins in Splicing	23
1.6.3 Miscellaneous Functional Contexts	24
1.7 Aim of the Work	25
1.7.1 GYF Domains Selected for Analysis.....	25
1.7.2 Approach.....	26

2	Selection Methods to Identify Protein–Protein Interactions.....	27
2.1	Endogenous Proteins from Cell Extracts.....	27
2.2	Expression of Proteins from DNA Libraries	27
2.2.1	<i>In Vivo</i> Selection	27
2.2.2	<i>In Vitro</i> Selection	28
2.3	Synthetic Peptides	29
3	NMR Experiments.....	31
3.1	Chemical Shift.....	32
3.2	Epitope Mapping and Determination of the Dissociation Constant	32
3.3	Backbone Assignment	33
4	Materials and Methods.....	35
4.1	Constructs	35
4.2	GYF Domain Constructs	39
4.2.1	Constructs for <i>In Vitro</i> Experiments.....	40
4.2.2	Bait and Prey Constructs.....	40
4.2.3	Fluorescence Protein Fusion Constructs	40
4.3	Constructs of Potential GYF Domain Interaction Partners	40
4.4	Library Construction	41
4.5	Bacterial Strains and Media.....	42
4.6	Protein Preparation.....	43
4.7	SPOT Analysis.....	43
4.8	Phage Display	44
4.9	Preparation of Peptides	44
4.10	NMR Titration Experiments	45
4.11	Fluorescence Titrations	46
4.12	Cell Culture and Lysis.....	46
4.12.1	Human Cell Lines.....	46
4.12.2	Yeast Cells	47
4.13	Transfection and Fluorescence Microscopy	47
4.14	GST Pulldown Assays	48
4.15	Yeast Two-Hybrid Analysis.....	49
4.16	Software.....	50
5	Establishment of the Experimental Setup.....	51
5.1	Expression of Functional GYF Domains	51
5.2	Binding Test of GYF Domains to Immobilized Peptides.....	53
5.3	Set up of Phage Display Experiments with GYF Domains	54

6	Recognition Sequences for the GYF Domain Reveal a Possible Spliceosomal Function of CD2BP2.....	59
6.1	Colocalization Studies of CD2BP2 and CD2 in HeLa Cells	67
7	Alternative Binding Modes of Proline-Rich Peptides Binding to the GYF Domain	69
8	Novel Interaction Partners of the CD2BP2-GYF Domain	83
8.1	Identification of Interaction Sites in Viral Proteins	91
9	GYF Domain Proteomics Reveals Interaction Sites in Known and Novel Target Proteins	93
9.1	Evaluation of Binding Motifs in Suggested Interaction Partners	111
10	Review: The GYF Domain.....	115
11	Discussion	129
11.1	Recognition Codes of GYF Domains.....	129
11.1.1	Definition of the Recognition Motifs	130
11.1.2	Evaluation of the Recognition Motifs.....	131
11.2	Functional Implications of GYF Domains	132
11.2.1	Involvement in CD2 Signaling.....	132
11.2.2	Spliceosomal Functions.....	132
11.2.3	Regulation of Translation Initiation	133
11.2.4	Other Possible Functions.....	134
11.3	Binding Models for the GYF Domain Subfamilies	135
11.3.1	Binding Modes for CD2BP2-type GYF Domains.....	136
11.3.2	Binding Mode for SMY2-type GYF Domains	137
11.4	Perspectives.....	138
11.4.1	Evolution of GYF Domains	138
11.4.2	Regulation of GYF Domain Functions	139
12	Appendix	145
12.1	Tables of Peptides, Synthesized on Membranes	145
12.2	NMR Backbone Assignment of the SMY2-GYF Domain.....	160
13	References	163
	Curriculum Vitae.....	187

