



Advanced Cell Biology & Biotechnology

# Biotechnology Project Lab

Giovanna Gambarotta  
& Isabella Tarulli

The lecture is about to begin....



# Biotechnology project

- identification of specific exons belonging to different NRG1 isoforms
- identification of exons and introns using BLAST and Ensemble
- use of Annhyb to design primers and to organize and edit sequences
- design primers for isoform specific expression analysis and for full length cDNA cloning
- design primers for quantitative gene expression analysis
- design primers for full length cDNA cloning (with additional restriction enzyme sites to facilitate cloning and subcloning)
- plan a reverse transcriptase (RT) reaction with positive and negative controls
- plan a polymerase chain reaction (PCR) with positive and negative controls
- use of Chromas to read an electropherogram
- plan cloning and subcloning from a vector to another vector
- restriction enzyme analysis and preparation of plasmid maps (with neb-cutter or other apps)
- blunting sticky ends
- project hybrid proteins fused with GFP
- project proteins with a FLAG tag
- solve some typical problems you can encounter in the laboratory like:
  - analyze relative/absolute quantitative real time PCR data
  - analyze protein quantification data
  - calculate the amount of cells you have to plate for a specific experiment
  - calculate the amount of ingredients in a reaction or to prepare a solution



# Biotechnology project

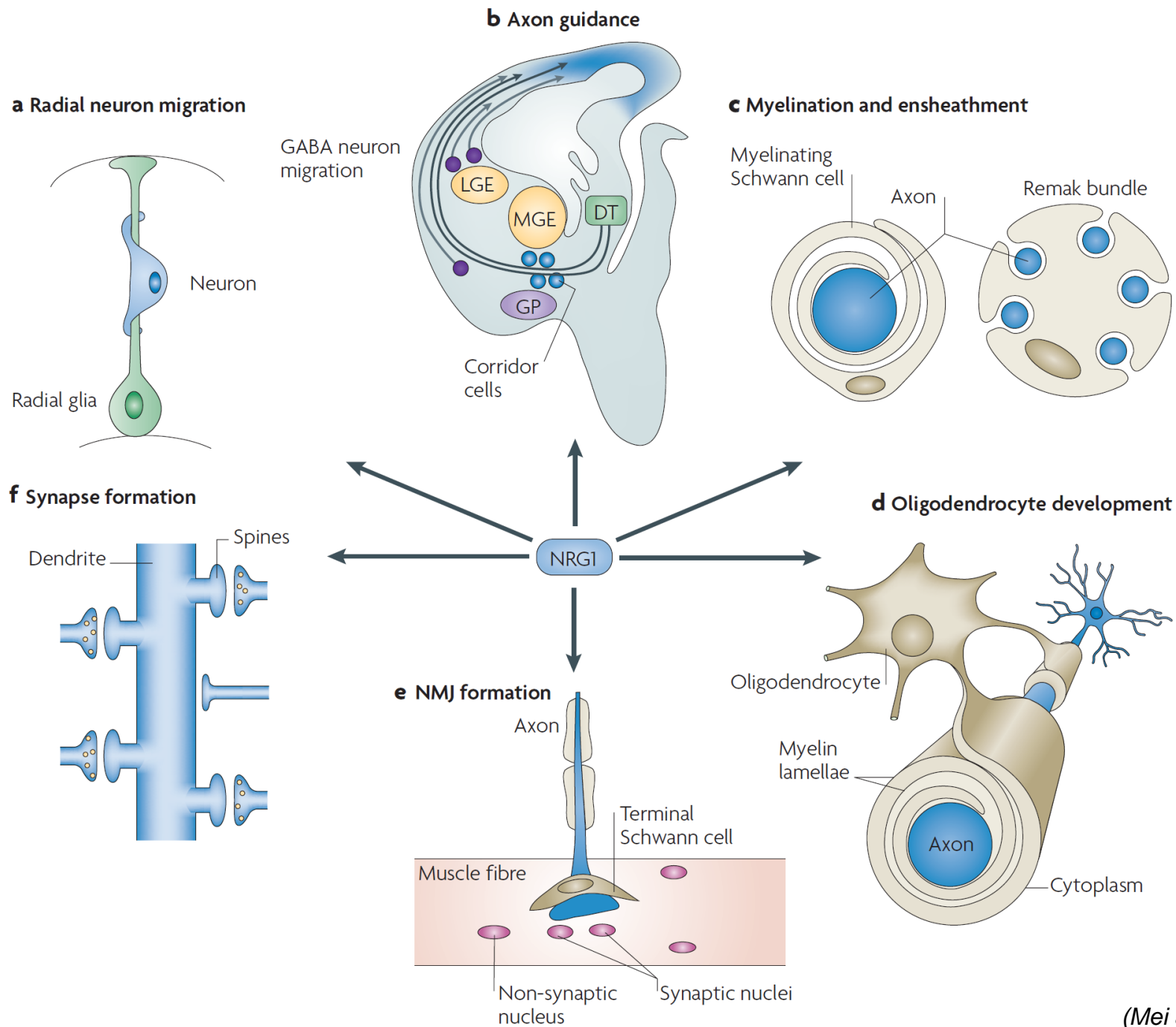
- **identification of specific domains belonging to different NRG1 isoforms**
- identification of exons and introns using BLAST and Ensemble
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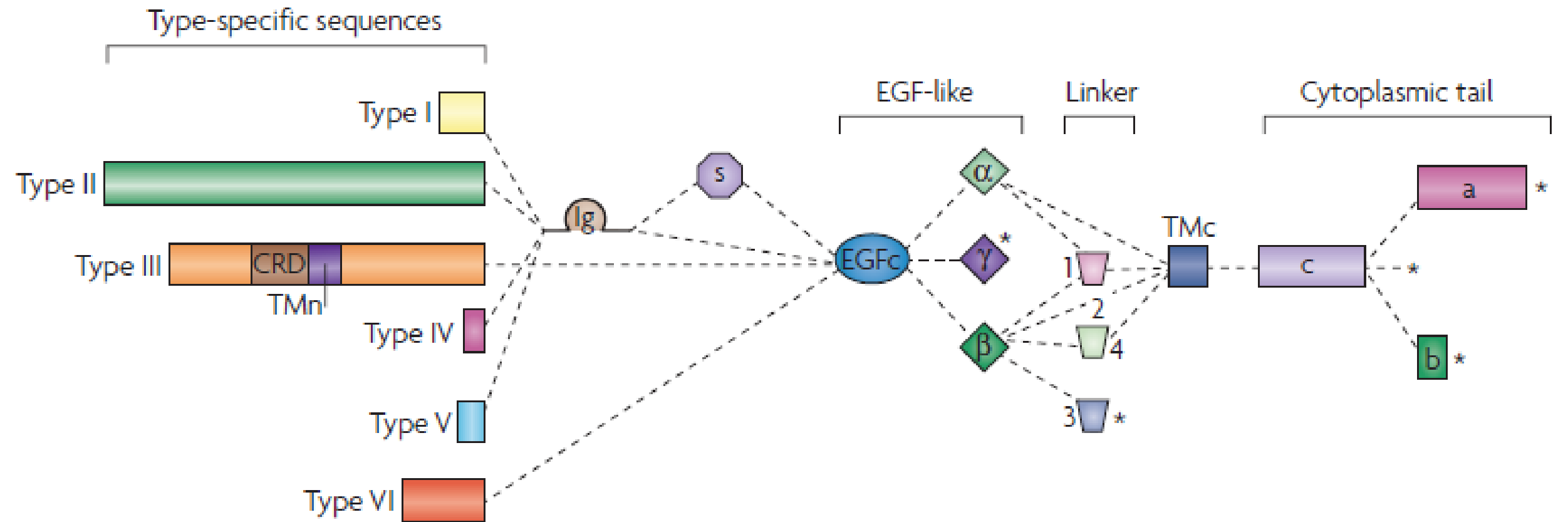


# Biotechnology project

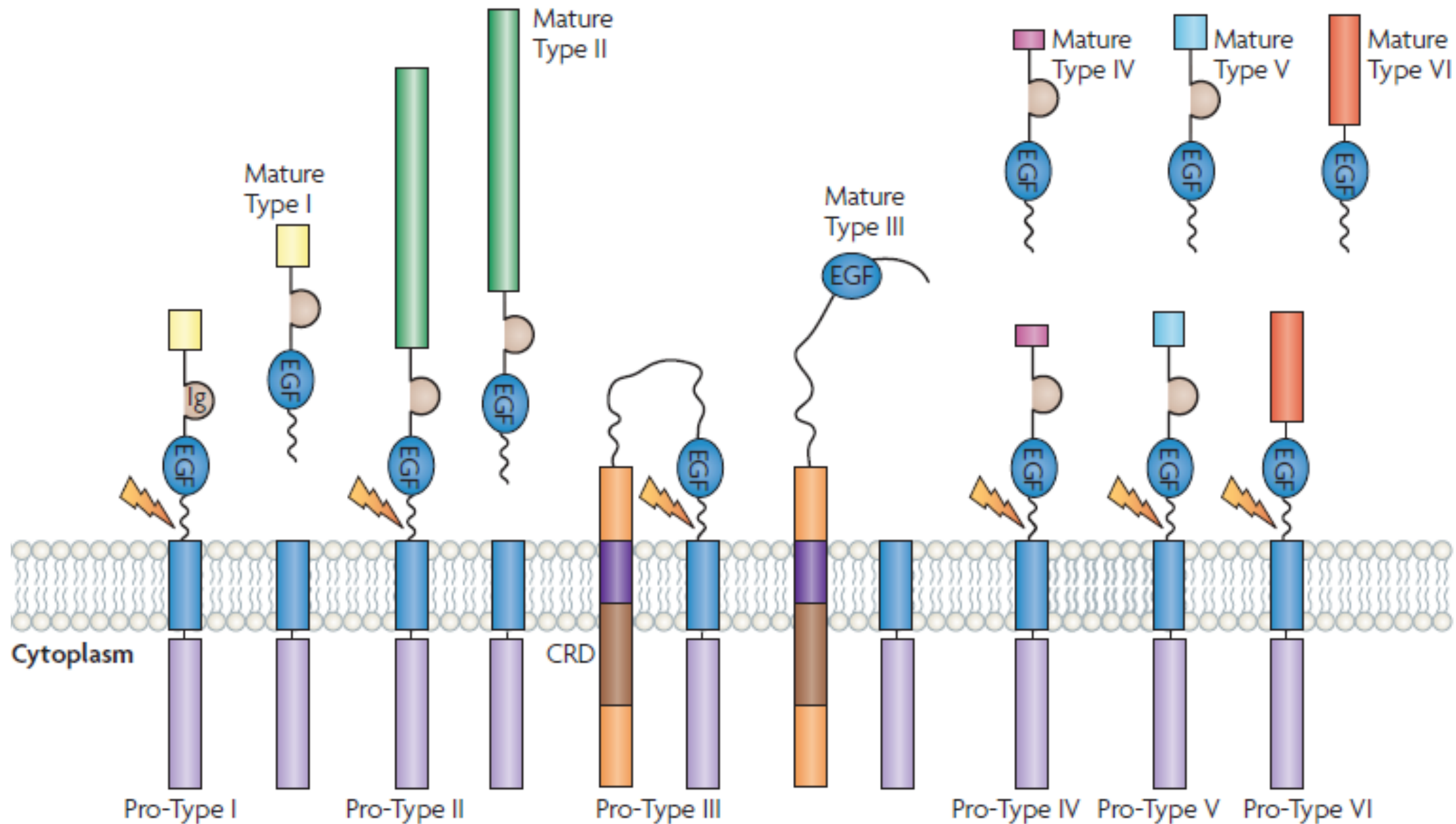
Today aims:

- 1-introduction on NRG1 and its the different isoforms
- 2-comparison of sequences to identify specific NRG1 domains
- 2-welcome test

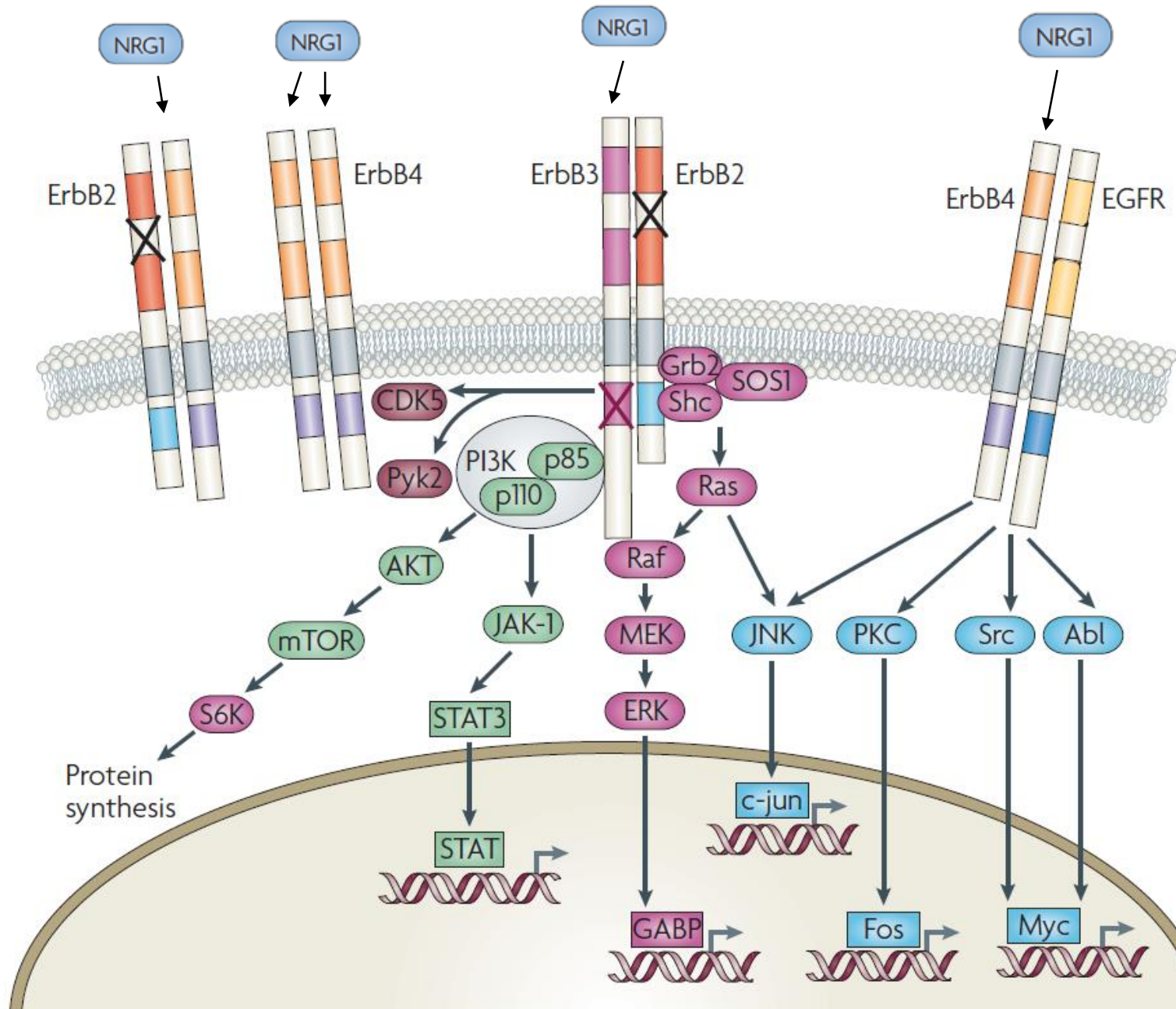




(Mei and Xiong, 2008)

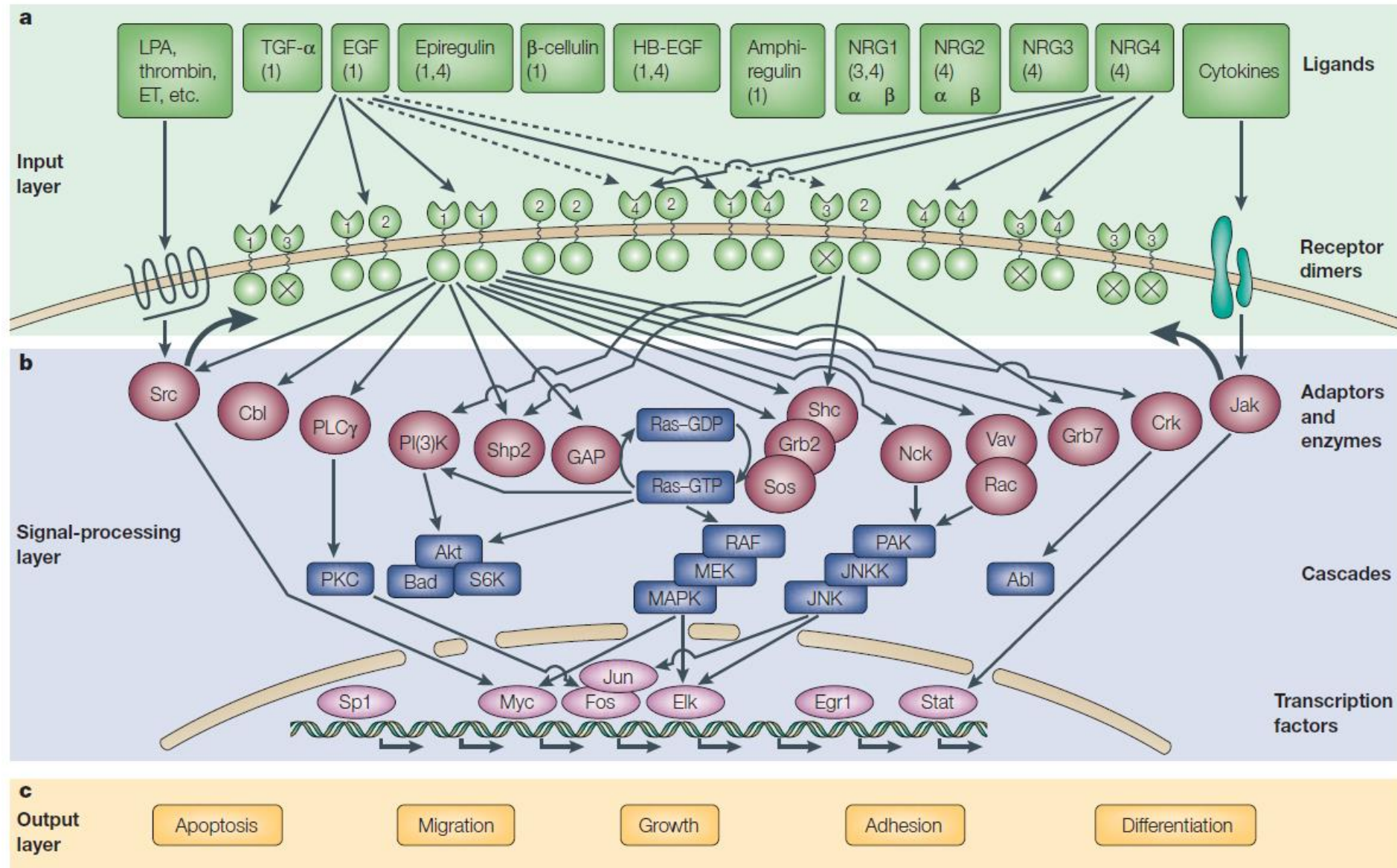


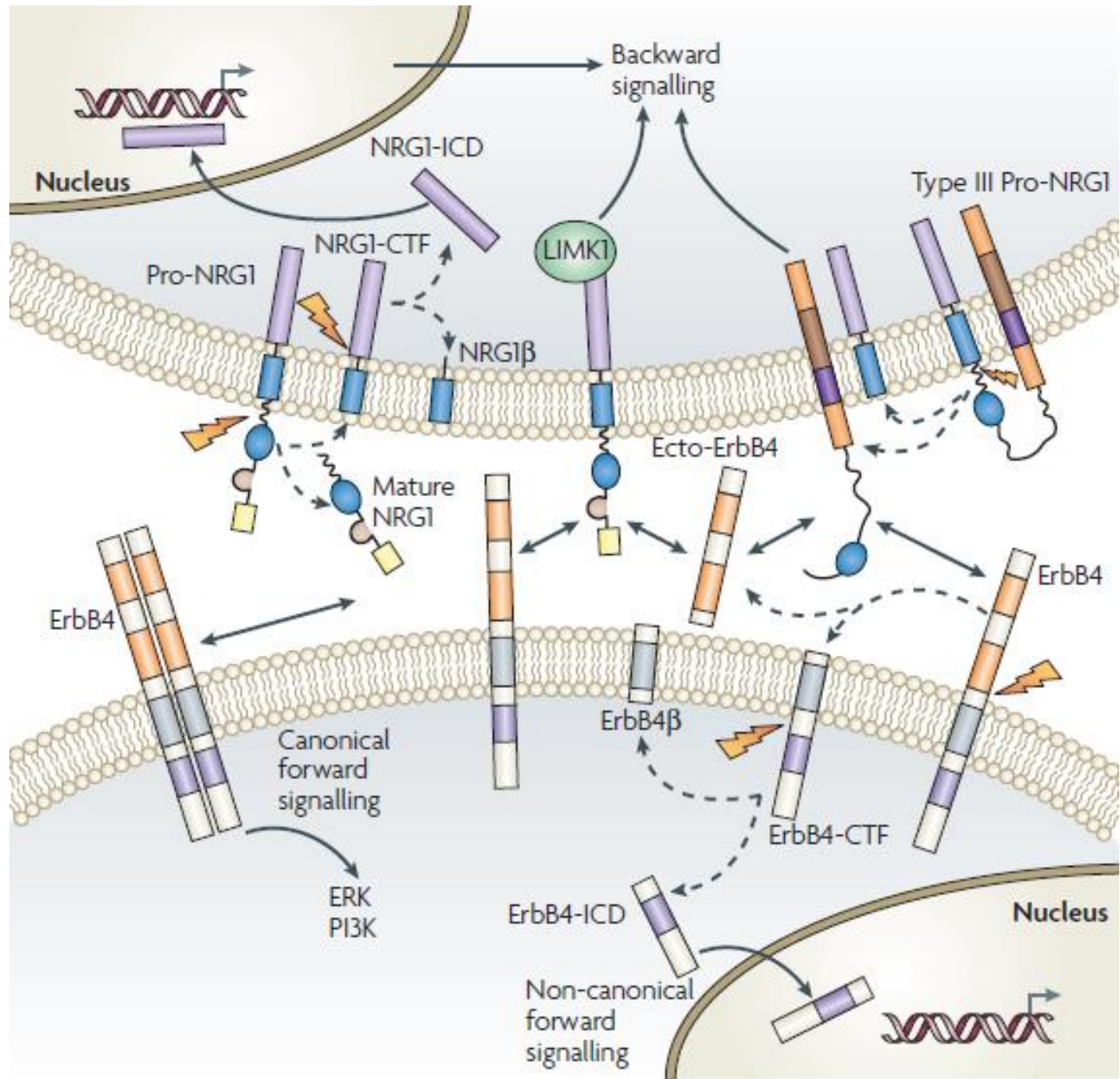
# NRG1 receptors belong to the ErbB receptor family





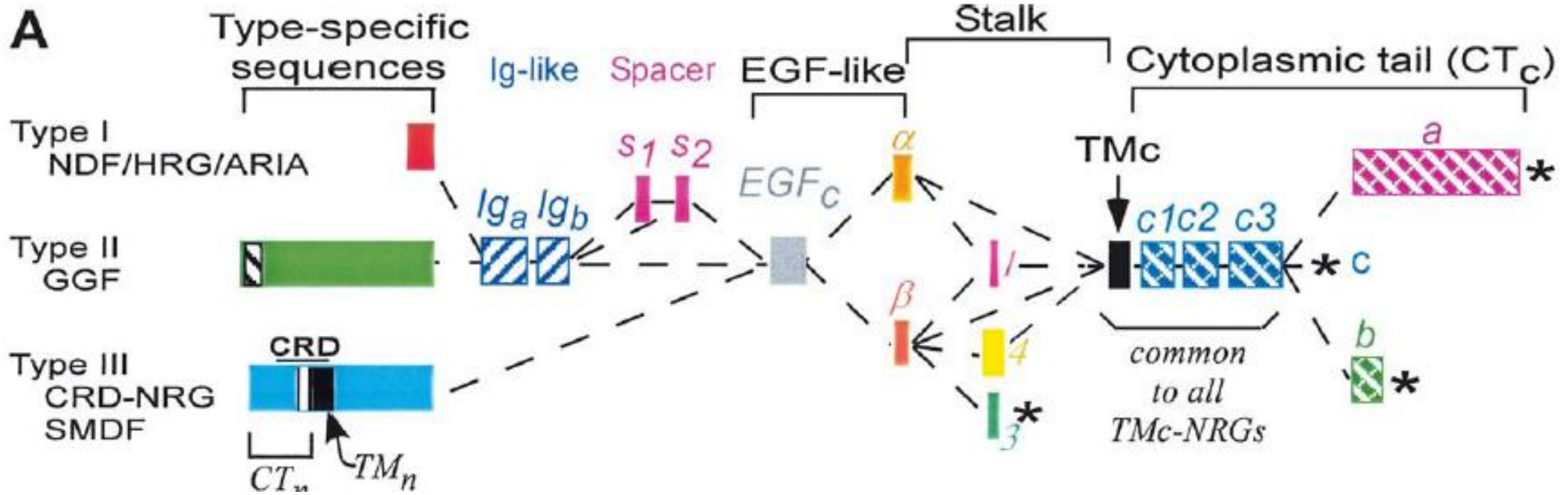
# The ErbB signalling network

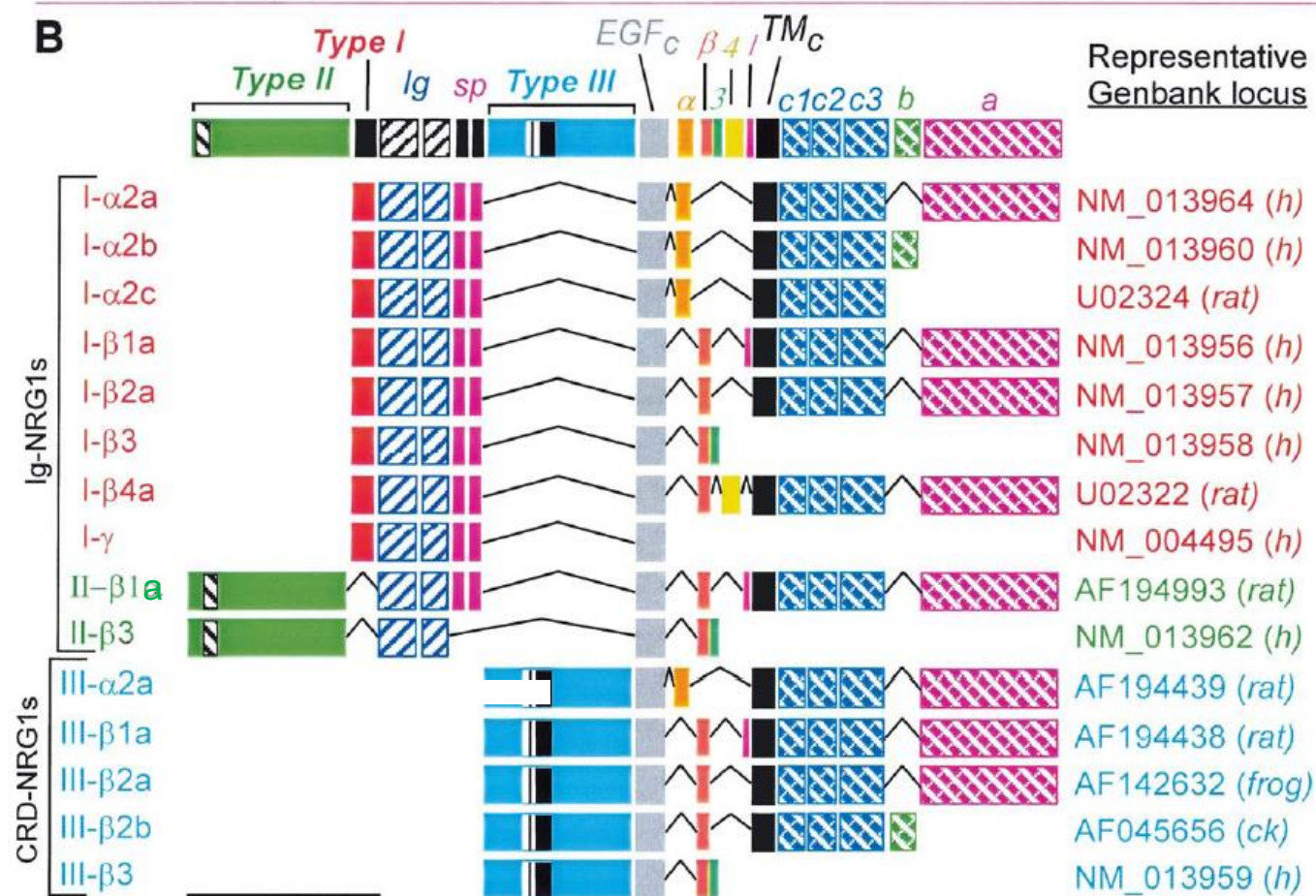
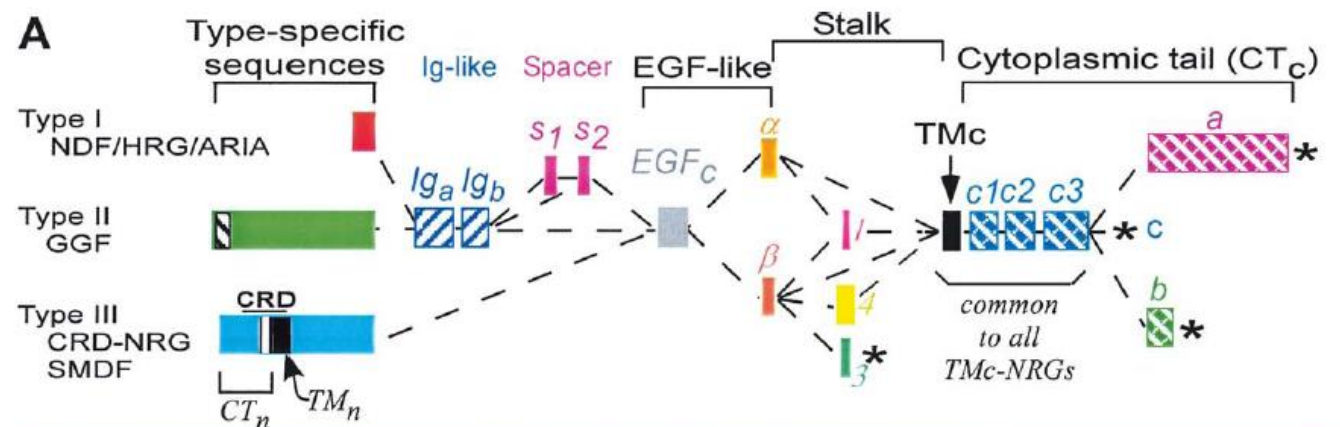


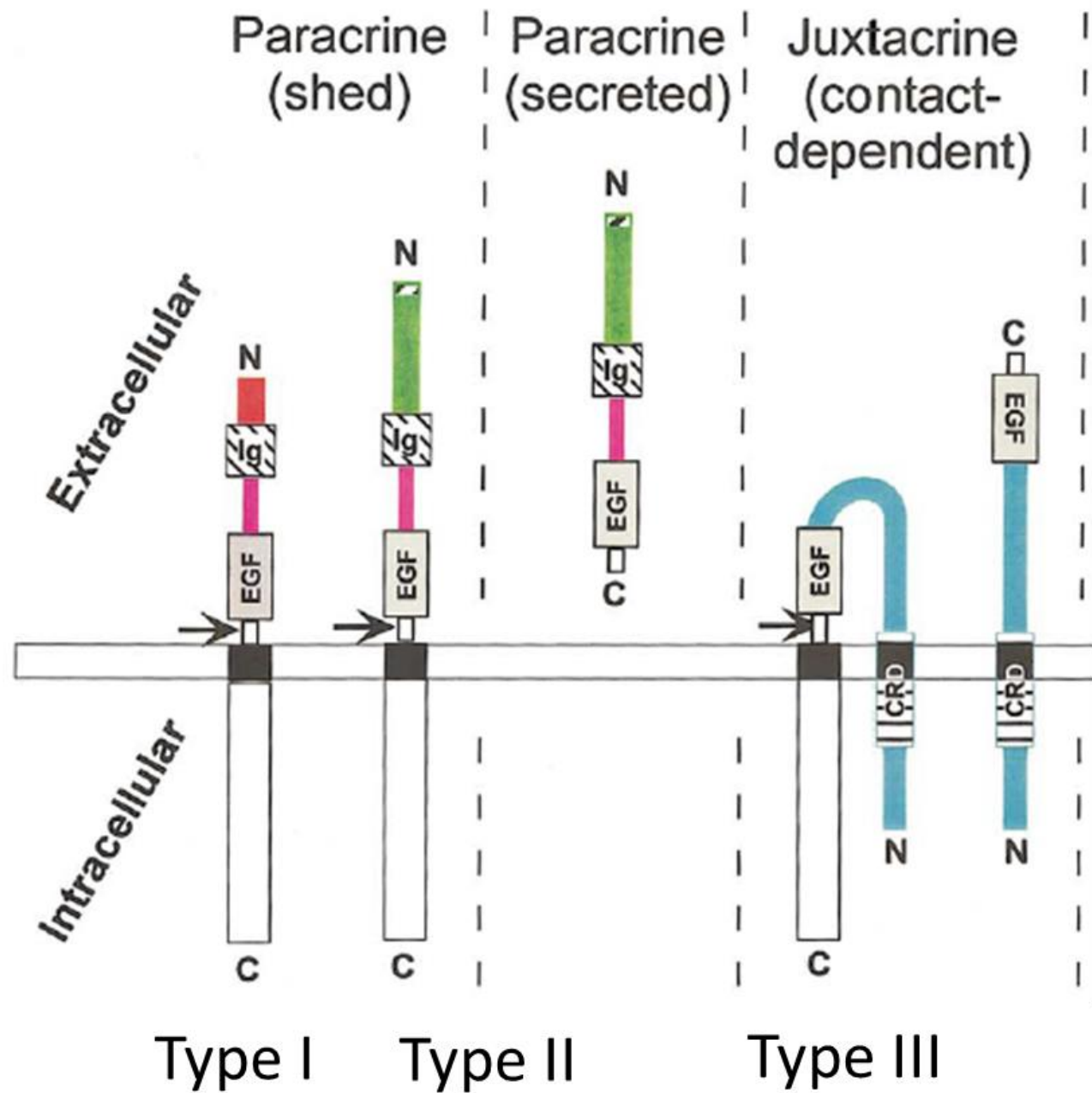


(Mei and Xiong, 2008)

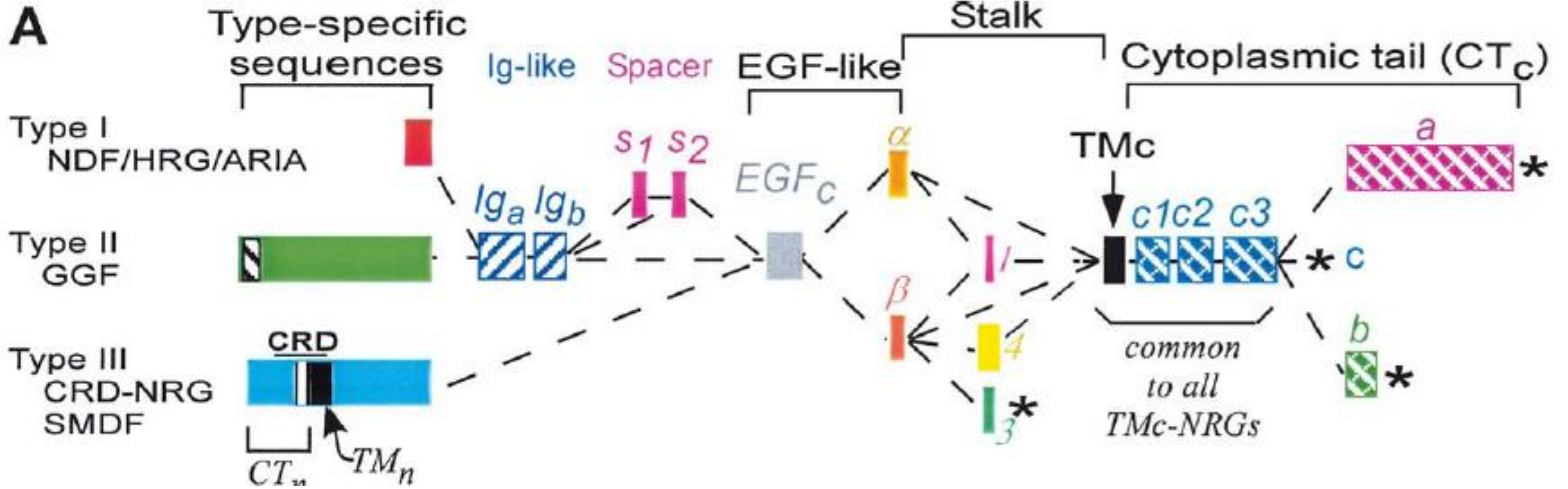
# NRG1 alternative splicing isoforms

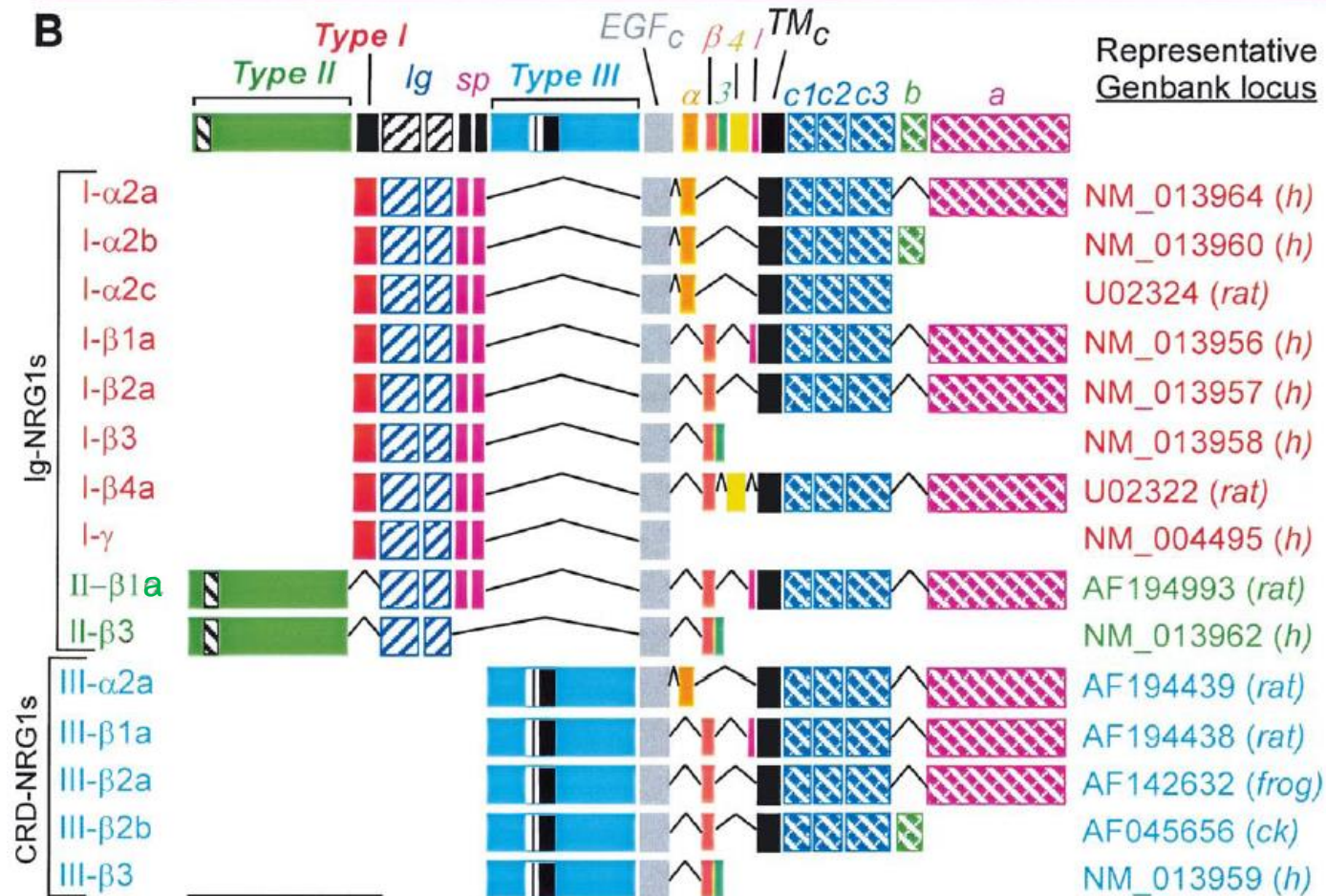
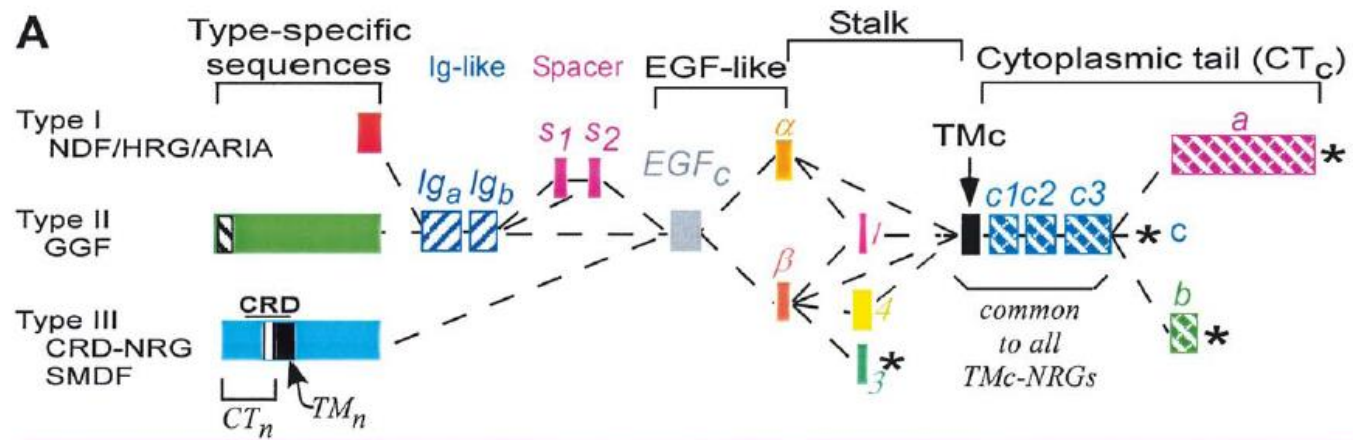




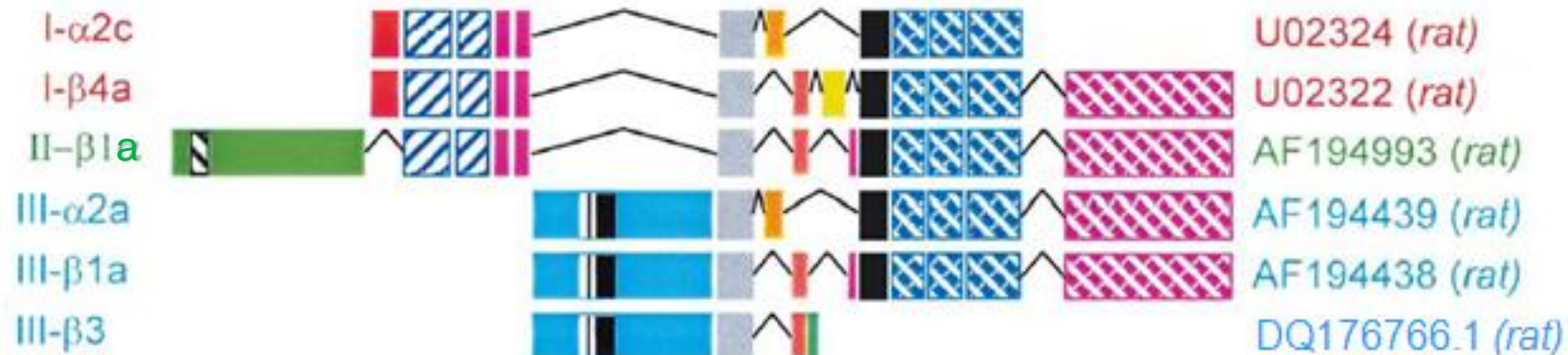
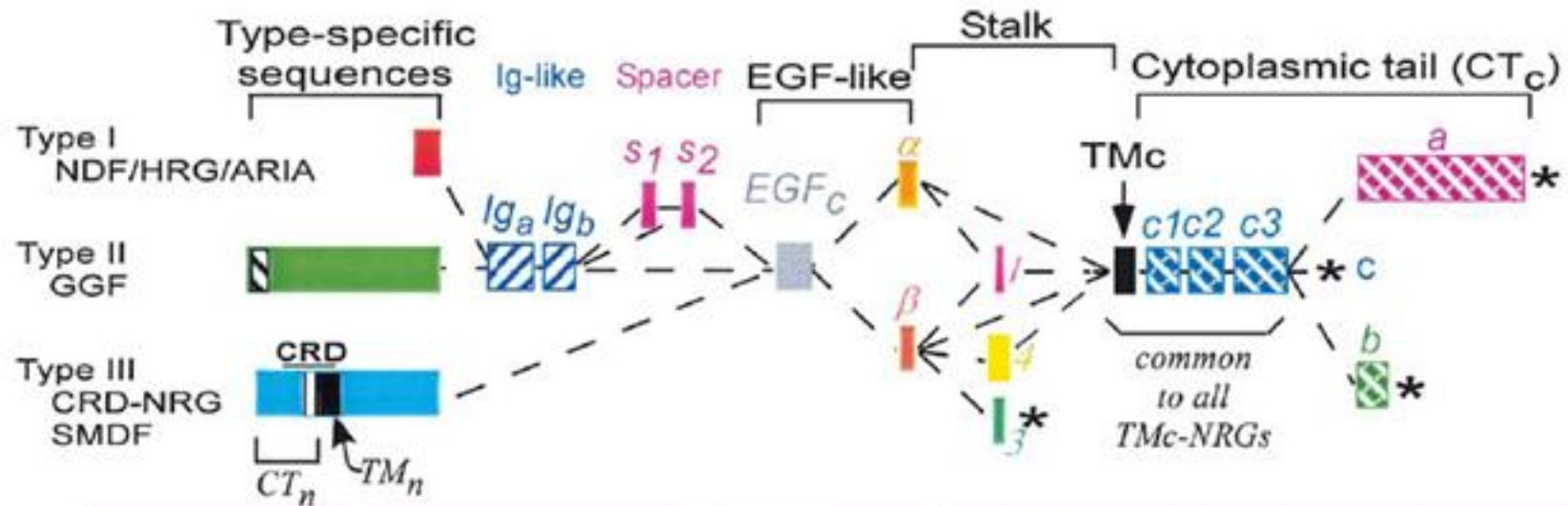


# NRG1 alternative splicing isoforms



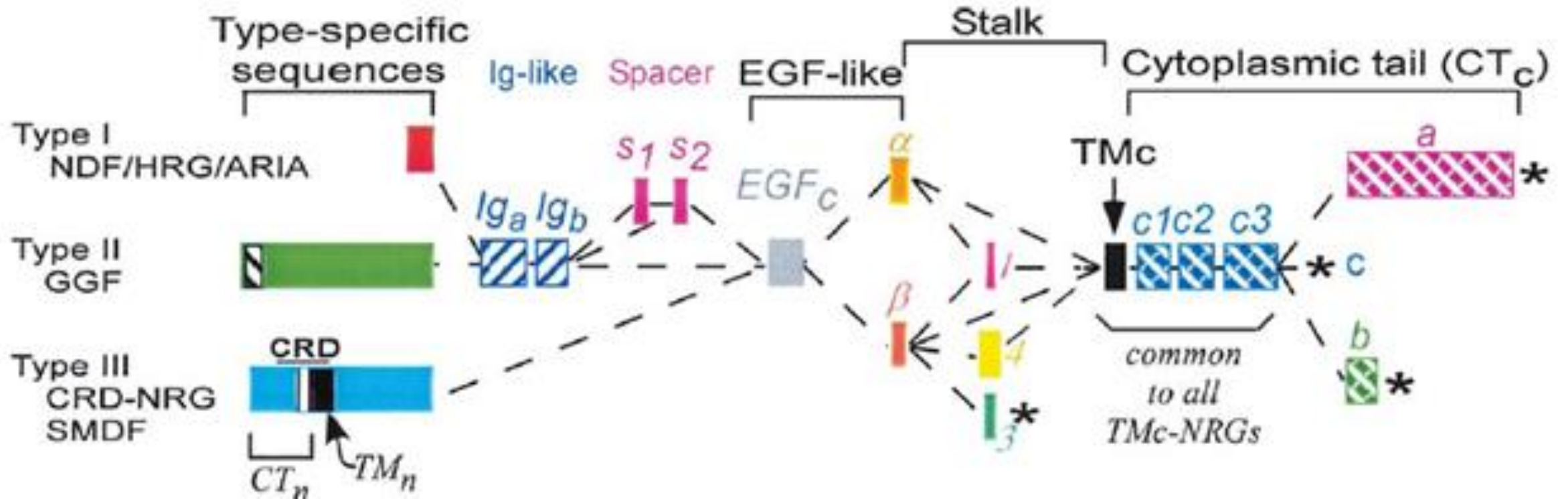


# rat NRG1 sequences



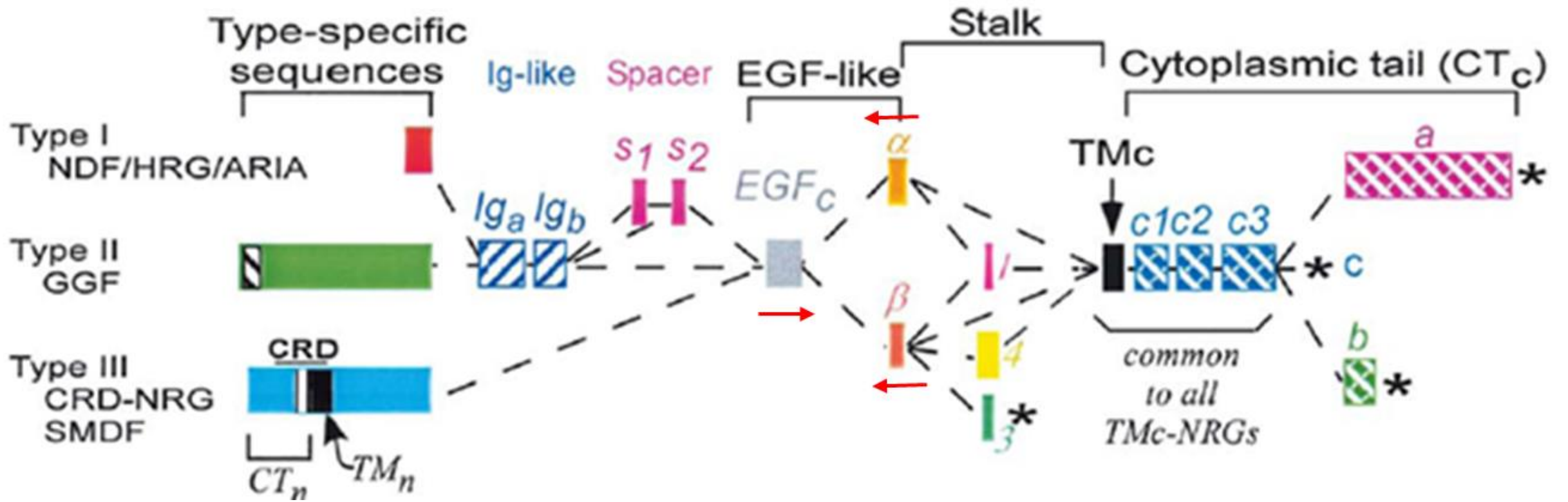


# NRG1 alternative splicing isoforms

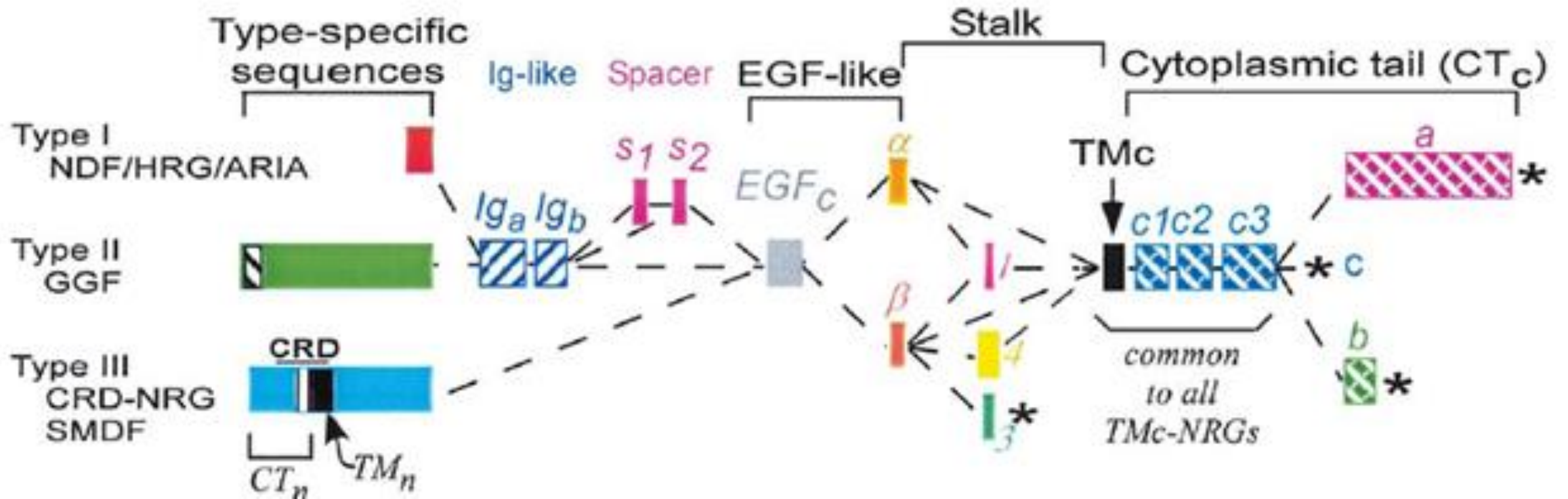


If you want to identify  **$\alpha$  and  $\beta$  isoforms**, where can you put primers for RT-PCR?

# $\alpha$ and $\beta$ isoforms

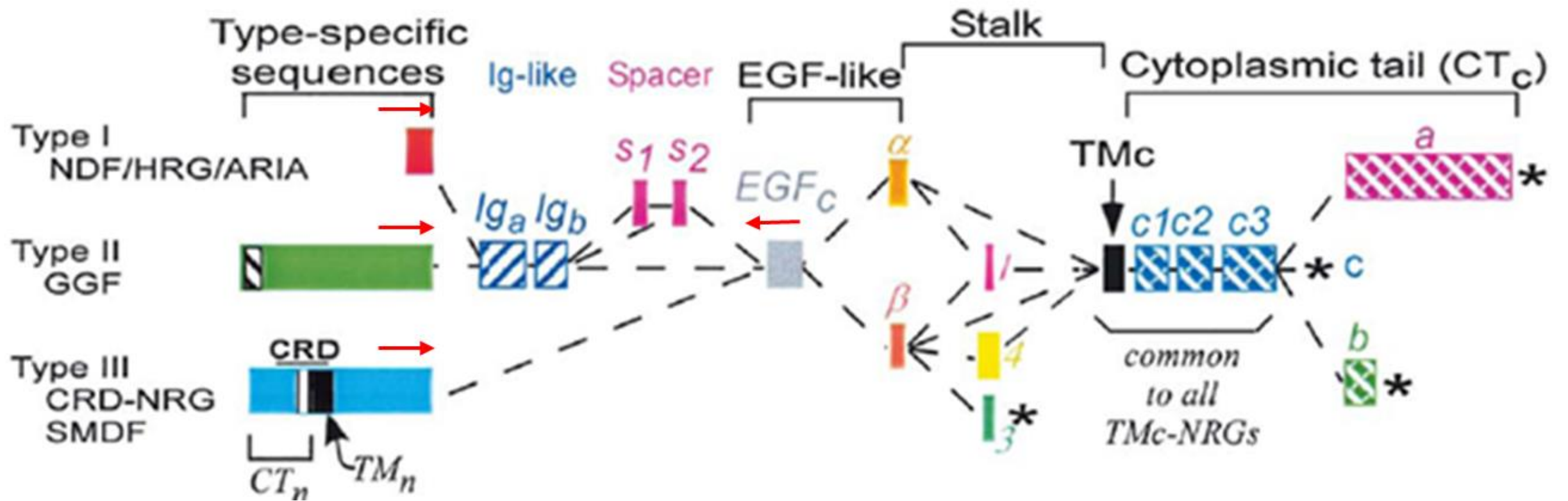


## NRG1 alternative splicing isoforms



If you want to identify **type I**, **type II** and **type III isoforms**, where can you put primers for RT-PCR?

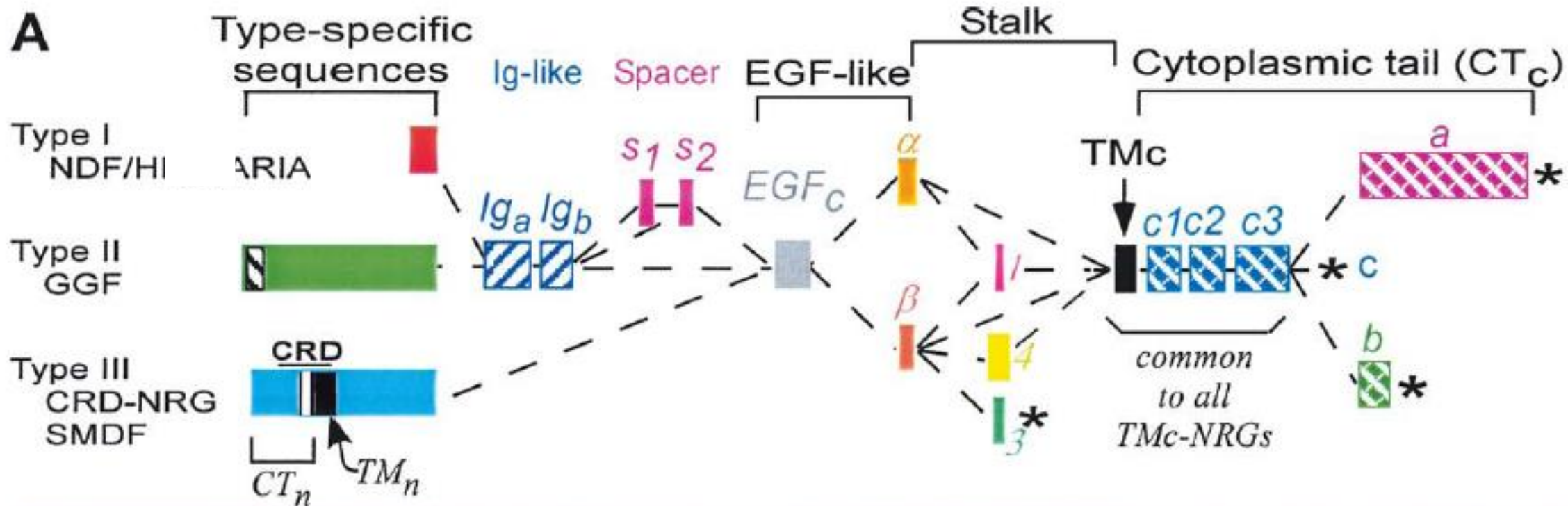
# Type I, II, III isoforms



## Preliminary study of NRG1 sequences to identify specific domains

- 1- download the three rat sequences of type III NRG1 from the ncbi site
- 2- copy only the coding sequences (form ATG to STOP) and save them in Annhyb (with name and code) or in a text/doc file.
- 3-pairwise compare the 3 different rat NRG1 type III sequences using BLAST to identify the sequences coding for  $\alpha$  and  $\beta$  domains.
- 4-load in moodle the sequences corresponding to  $\alpha$  and  $\beta$  domains.
- 5- compare the different rat sequences to identify the regions corresponding to the **EGF-like** domain, **type I**, **type II**, **type III** domains.

I aim: identify  $\alpha$  and  $\beta$  domains to design primers for RT-PCR.



<http://www.ncbi.nlm.nih.gov/>

NCBI Resources How To Sign in to NCBI

Nucleotide af194439 Search

National Center for Biotechnology Information

COVID-19 is an emerging, rapidly evolving situation.  
Get the latest public health information from CDC: <https://www.coronavirus.gov>.  
Get the latest research from NIH: <https://www.nih.gov/coronavirus>.  
Find NCBI SARS-CoV-2 literature, sequence, and clinical content: <https://www.ncbi.nlm.nih.gov/sars-cov-2/>.

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Variation

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[SNP](#)  
[Gene](#)  
[Protein](#)  
[PubChem](#)

**NCBI News & Blog**  
The latest in COVID-19 related human gene annotation now in NCBI RefSeq and Gene  
02 Oct 2020  
Interacted in human genes involved in

# Rattus norvegicus SMDF neuregulin alpha 2a (Nrg1) mRNA, complete cds, alternatively spliced

GenBank: AF194439.1

[FASTA](#) [Graphics](#)

Go to:

LOCUS AF194439 2540 bp mRNA linear ROD 01-NOV-2000  
DEFINITION Rattus norvegicus SMDF neuregulin alpha 2a (Nrg1) mRNA, complete cds, alternatively spliced.  
ACCESSION AF194439  
VERSION AF194439.1  
KEYWORDS -  
SOURCE Rattus norvegicus (Norway rat)  
ORGANISM [Rattus norvegicus](#)  
Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Rattus.  
REFERENCE 1 (bases 1 to 2540)  
AUTHORS Carroll,S.L., Anderson,K.D. and Frohnert,P.W.  
TITLE Structural and Functional Diversity of SMDF Neuregulin Splice Variants Expressed in the Adult Rat Nervous System  
JOURNAL Unpublished  
REFERENCE 2 (bases 1 to 2540)  
AUTHORS Carroll,S.L., Anderson,K.D. and Frohnert,P.W.  
TITLE Direct Submission  
JOURNAL Submitted (13-OCT-1999) Pathology, The University of Alabama at Birmingham, 1720 Seventh Avenue South, SC843, Birmingham, AL 35294-0017, USA  
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/gene="Nrg1"  
/note="sensory and motor neuron-derived factor splice variants expressed in the adult rat nervous system"

Customize view

## Analyze this sequence

Run BLAST

Pick Primers

Highlight Sequence Features

Find in this Sequence

## Articles about the Nrg1 gene

Neuregulin-1 impacting bone marrow mesenchymal stem cell mi [Mol Med Rep. 2019]

Neuregulin-1 $\beta$  attenuates sepsis-induced diaphragm atrop [J Muscle Res Cell Motil. 2019]

Neuregulin-1 protects cardiac electrical conduction through downregul [Pharmazie. 2019]

See all...

## Pathways for the Nrg1 gene

Downregulation of ERBB2 signaling

RET signaling

EGFR tyrosine kinase inhibitor resistance

See all...

## Reference sequence information

RefSeq alternative splicing

See 21 reference mRNA sequence splice variants for the Nrg1 gene.

## More about the Nrg1 gene



→ ↻ 🏠 <https://www.ncbi.nlm.nih.gov/nucore/af194439>

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SSNSESETEDERVGEDTPFLGIQNPLAASLEVAPAFRLAESRTNPAGRFSTQEELQAR
LSSVIANQDPIAV"
```

[gene](#)  
**CDS**

You can copy all the sequence, writing in the description where ATG and STOP are, or you can copy only the coding sequence.

For today aim, copy only the coding sequence.

coding sequence



## LSSVIANQDPIAV"

ORIGIN

```

1 gaattcggca cgaggcggca gcttgcttcc tattttggtc ccttgccttc ttgaccaacc
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961 accctgagg tgagaacacc caagtcagga actcagccac aaacaacaga aactaacctg
1021 caaactgctc ctaaactttc cacatcaaca tccacgactg ggaccagcca tctcataaag
1081 tgtgcggaga aggagaaaac tttctgtgtg aatgggggcg agtgcttcac ggtgaaggac
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1381 cttcggtcag aacggagcaa cctgggtgaa atagcgaatg ggcctacca cccaaaccg
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1501 catattggtg agagagaagt ggagacttcc ttttccacca gtcattacac ttccacagcc
1561 catcactcca cgactgtcac ccagactcct agtcacagct ggagtaatgg gcacacggag
1621 agcgtcattt cagaaagcaa ctccgtaatc atgatgtctt cggtagagaa cagcaggcac

```

This is the coding sequence (CDS) only. You have to SELECT it, copy and paste.

## ORIGIN

```
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61 cggcatgggt tggagaagca tttgaaagaa ctgaaaaagt gtcccagaaa caacagctca
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961 accctgagg tgagaacacc caagtcagga actcagccac aaacaacaga aactaacctg
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1081 tgtgcggaga aggagaaaac tttctgtgtg aatggggggcg agtgcttcac ggtgaaggac
```

You have to SELECT it, copy and paste in Annhyb, or in doc/text document.

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GRAF, a new tool and closely related genomic dataset

[Genome-wide analysis](#)  
[Genome Workbench](#)

Recent updates to  
Workbench include

# BLAST®

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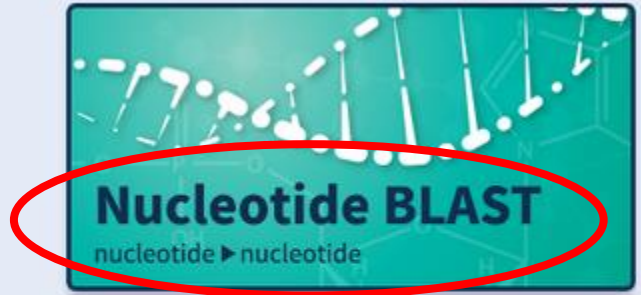
## Basic Local Alignment Search Tool

**BLAST** finds regions of similarity between biological sequences. The program compares nucleotide or protein sequences to sequence databases and calculates the statistical significance. [Learn more](#)

**NEWS**

**Magic-BLAST 1.3.0 released**  
A new version of the BLAST RNA-seq mapping tool is now available.  
Thu, 28 Sep 2017 16:00:00 EST [More BLAST news...](#)

## Web BLAST



**Nucleotide BLAST**  
nucleotide ▶ nucleotide



**blastx**  
translated nucleotide ▶ protein



**tblastn**  
protein ▶ translated nucleotide



**Protein BLAST**  
protein ▶ protein

## BLAST Genomes

**Search**

- Human
- Mouse
- Rat
- Microbes

Standard Nucleotide BLAST

blastn blastp blastx tblastn tblastx

Enter Query Sequence

BLASTN programs search nucleotide databases using a nucleotide query. more...

Reset page Bo

Enter accession number(s), gi(s), or FASTA sequence(s)

Clear

Query subrange

Text input field for accession numbers or FASTA sequences.

From input field for query subrange.

To input field for query subrange.

Or, upload file

Sfoggia...

Nessun file selezionato.

Job Title

Text input field for job title.

Enter a descriptive title for your BLAST search

Align two or more sequences

Choose Search Set

Database

Human genomic + transcript Mouse genomic + transcript Others (nr etc.):

Nucleotide collection (nr/nt)

Organism

Optional

Enter organism name or id--completions will be suggested

Exclude

Enter organism common name, binomial, or tax id. Only 20 top taxa will be shown

Exclude

Optional

Models (XM/XP) Uncultured/environmental sample sequences

Limit to

Optional

Sequences from type material

Entrez Query

Optional

Text input field for Entrez query.

YouTube Create custom database

Enter an Entrez query to limit search

Program Selection

Optimize for

- Highly similar sequences (megablast)
More dissimilar sequences (discontiguous megablast)
Somewhat similar sequences (blastn)

https://blast.ncbi.nlm.nih.gov/Blast.cgi?PAGE=MegaBlast&PROGRAM 67% Cerca

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**BLAST** » blastn suite Home Recent Results Saved Strategies

### Align Sequences Nucleotide BLAST

blastn blastp blastx tblastn tblastx

BLASTN programs search nucleotide subjects using a nucleotide query [more...](#) [Reset page](#) [Bookmark](#)

**Enter Query Sequence**

Enter accession number(s), gi(s), or FASTA sequence(s) [clear](#) Query subrange [From](#) [To](#)

Or, upload file [Sfoggia...](#) Nessun file selezionato.

Job Title  Enter a descriptive title for your BLAST search

Align two or more sequences

**Enter Subject Sequence**

Enter accession number(s), gi(s), or FASTA sequence(s) [clear](#) Subject subrange [From](#) [To](#)

Or, upload file [Sfoggia...](#) Nessun file selezionato.

**Program Selection**

Optimize for

- Highly similar sequences (megablast)
- More dissimilar sequences (discontiguous megablast)
- Somewhat similar sequences (blastn)

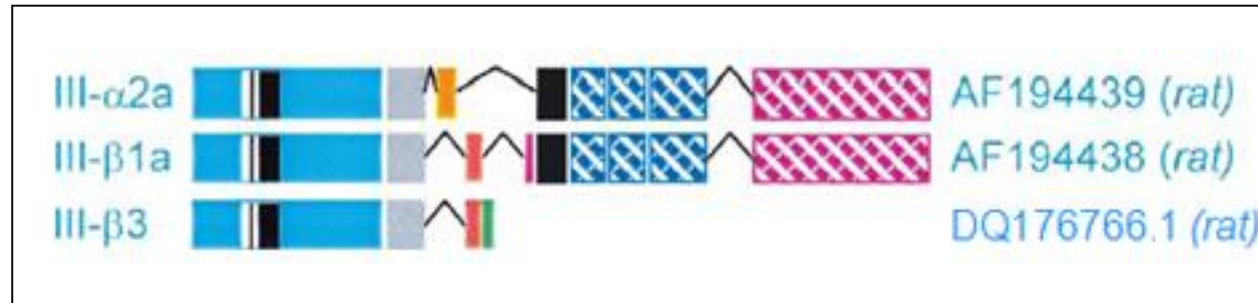
Choose a BLAST algorithm

**BLAST** Search nucleotide sequence using Megablast (Optimize for highly similar sequences)

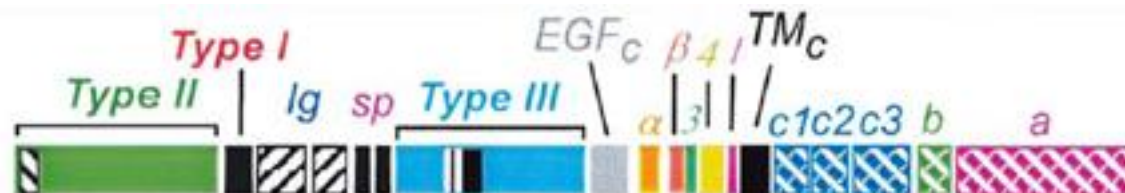
Show results in a new window

[Algorithm parameters](#)

## Three rat NRG1 type III sequences

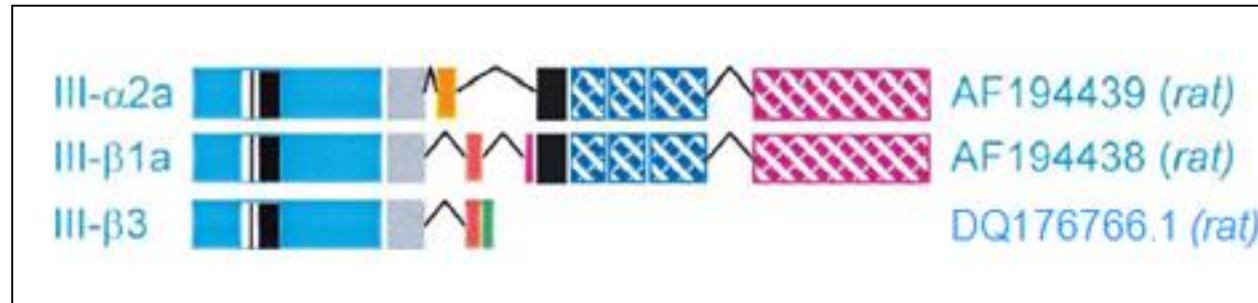


- When you compare AF194439 with AF194438, the **NON ALIGNED** parts correspond to
- the domain in the AF194439 sequence
  - the domain in the AF194438 sequence

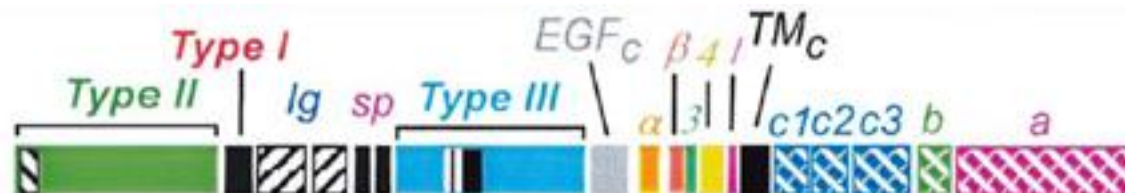




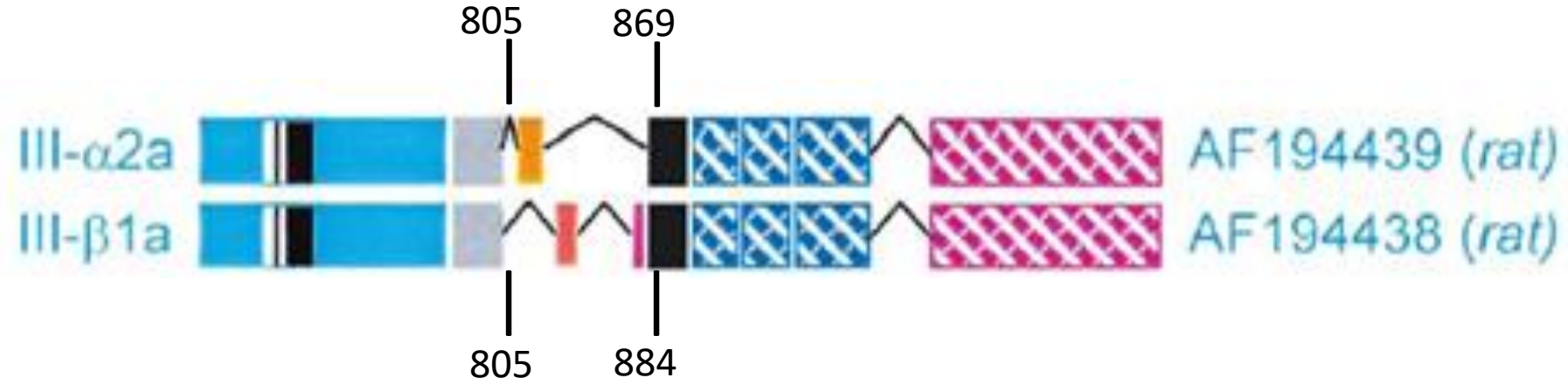
## Three rat NRG1 type III sequences



- When you compare AF194439 with AF194438, the **NON ALIGNED** parts correspond to
- the  $\alpha$  domain in the AF194439 sequence
  - the  $\beta$ 1 domains in the AF194438 sequence



An example to help you:



```

Query 661 CCTAAACTTTCCACATCAACATCCACGACTGGGACCAGCCATCTCATAAAGTGTGCGGAG 720
Sbjct 661 CCTAAACTTTCCACATCGACATCCACGACTGGGACCAGCCATCTCATAAAGTGTGCGGAG 720

Query 721 AAGGAGAAAACCTTTCTGTGTGAATGGGGCGAGTGCTTCACGGTGAAGGACCTGTCAAAC 780
Sbjct 721 AAGGAGAAAACCTTTCTGTGTGAATGGGGCGAGTGCTTCACGGTGAAGGACCTGTCAAAC 780

Query 781 CCGTCAAGATACTTGTGCAAGTGCC 805
Sbjct 781 CCGTCAAGATACTTGTGCAAGTGCC 805
  
```

Range 2: 884 to 2103 [Graphics](#)    ▼ Next Match    ▲ Previous Match    ▲ First Match

Score	Expect	Identities	Gaps	Strand
2254 bits(1220)	0.0	1220/1220(100%)	0/1220(0%)	Plus/Plus

```

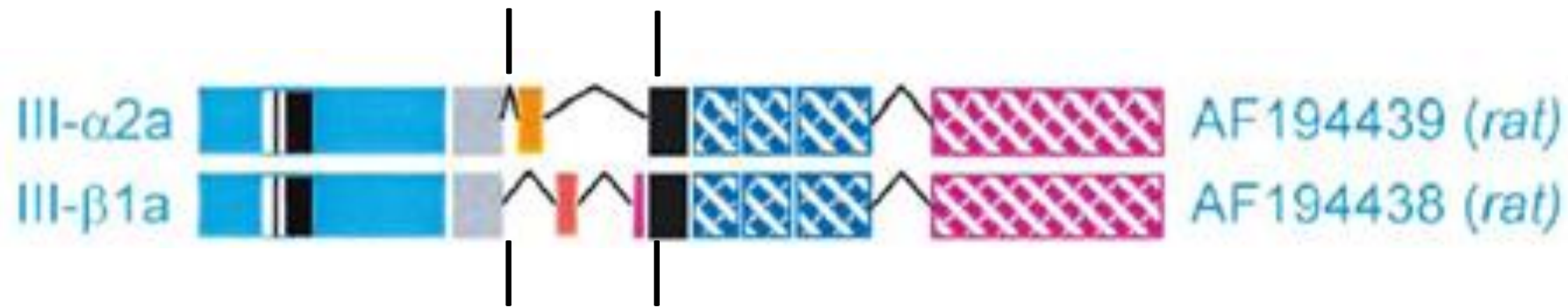
Query 869 AAGCGGAGGAACTCTACCAGAAGAGGGTGTGACAATTACTGGCATCTGTATCGCCCTGC 928
Sbjct 884 AAGCGGAGGAACTCTACCAGAAGAGGGTGTGACAATTACTGGCATCTGTATCGCCCTGC 943

Query 929 TGGTGGTCGGCATCATGTGTGTGGTGGCCTACTGCAAAACCAAGAAGCAGCGGCAGAAGC 988
Sbjct 944 TGGTGGTCGGCATCATGTGTGTGGTGGCCTACTGCAAAACCAAGAAGCAGCGGCAGAAGC 1003

Query 989 TTCATGATCGGCTTCGGCAGAGTCTTCGGTCAGAACGGAGCAACCTGGTGAACATAGCGA 1048
Sbjct 1004 TTCATGATCGGCTTCGGCAGAGTCTTCGGTCAGAACGGAGCAACCTGGTGAACATAGCGA 1063

Query 1049 ATGGGCCTCACCACCCAAACCCGCCAGAGAACGTGCAGCTGGTGAATCAATACGTAT 1108
  
```

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When you compare AF194439 with AF194438, the **NON ALIGNED** parts correspond to

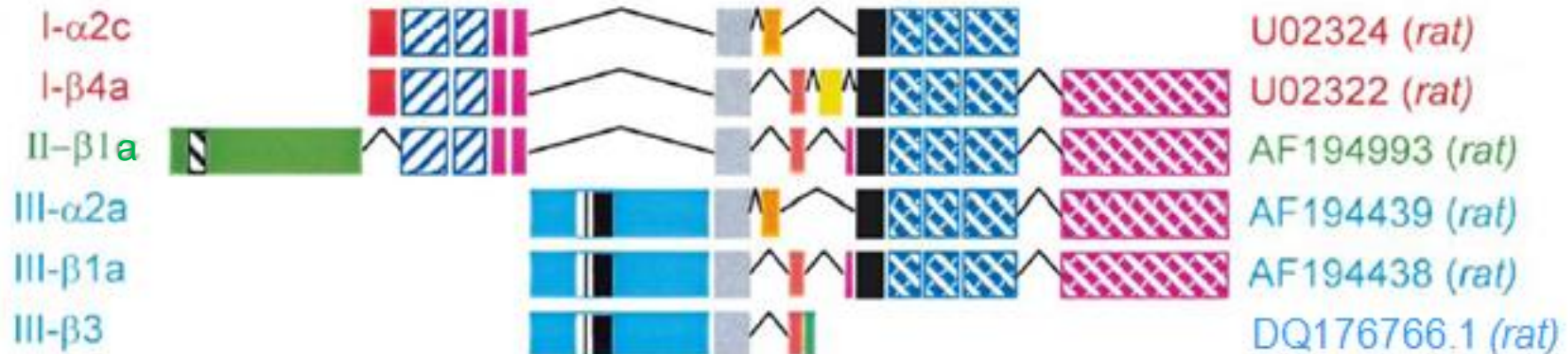
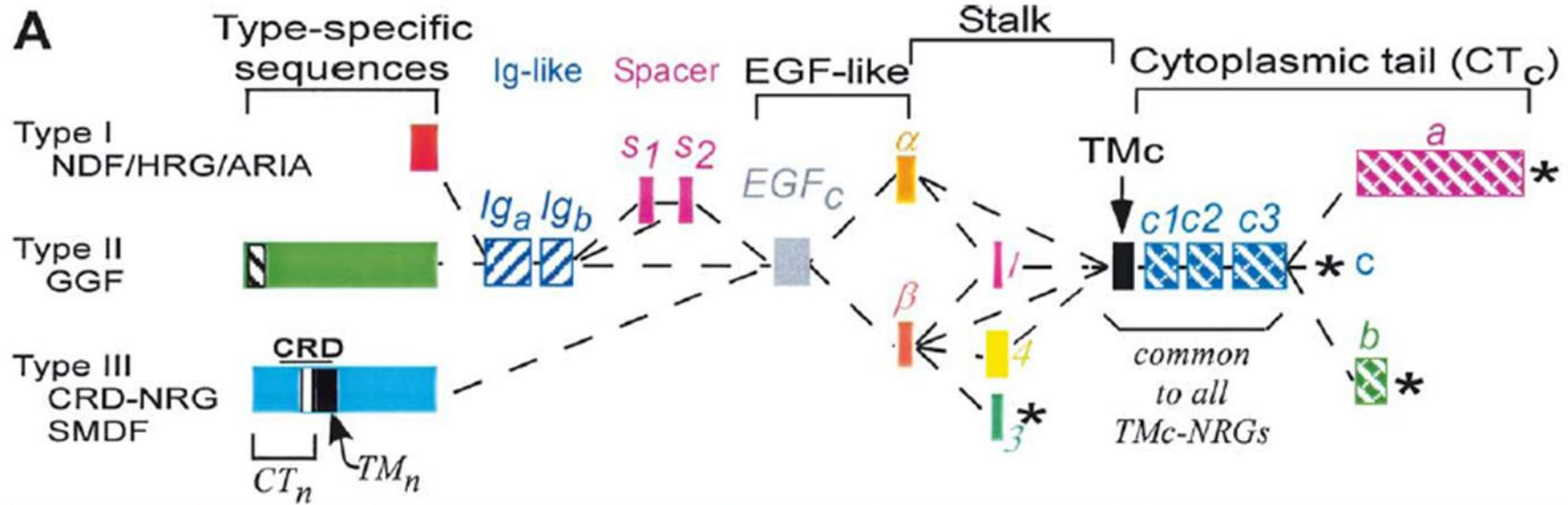
- the  $\alpha$  domain in the AF194439 sequence
- the  $\beta$ 1 domains in the AF194438 sequence



When you compare AF194438 with DQ176766, the **ALIGNED** parts correspond to **-type I-EGF-like-beta** domains in both sequences

- Where does domain  $\beta$  end?
- Where does domain 1 start?
- Where does domain 3 start?

II aim: identify EGF-like, **type I**, **II**, **III** domains



- upload all information corresponding to  $\alpha$ ,  $\beta$ , EGF-like domain, type I, type II and type III domains in your moodle page.

I will not correct all your single activities.  
I will check only a few, chosen at random.

I will upload the correct answers and you will have to verify the correctness of your results.