Deciphering the intricate role of intrinsically disordered regions in transcription factor regulation

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Disclosures

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Thanks to Prof. Gilbert Reibnegger and Amtsrat Gerhard Ledinski
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#madllab on Instagram and Twitter
Hallmarks of Ageing

Problems with DNA repair (genomic instability)

Problems with garbage disposal (loss of proteostasis)

Problems with cellular switches (epigenetic alterations)
Hallmarks of Ageing

- Deregulated nutrient sensing
- Distorted cell-cell communication
- Genomic instability
- Telomere shortening
- Epigenetic alterations
- Loss of proteostasis
- Mitochondrial dysfunction
- Cellular senescence
- Stem cell exhaustion

References:
Disordered proteins are key players in ageing

Our vision – deciphering and targeting key molecular mechanisms of ageing

**Wnt signalling and intersecting pathways**
- Signalling
- Transcription
- FOXO/p53/Wnt pathways
- Phase separation
- Misregulation in human diseases

**Regulation of RNA-binding proteins**
- RG/RGG
- PTMs
- Co-factors
- Nucleus

**Disordered proteins in health and disease**

**Metabolomics**
- Clinical
- Pre-clinical

**Method development**
- Rosetta
- Solvent PREs
- Integrative modeling

23.06.2023
Intrinsically disordered proteins/regions have unique properties

- Post-translational modifications (PTMs): phosphorylation, methylation, acetylation, ...
- Mutations (e.g. cancer, neurodegeneration)
- Binding of folded co-factors
- Residual structure
- Multimerization, LLPS
Intrinsically disordered proteins/regions are highly modified

Post-translational modifications (PTMs)
- phosphorylation
- methylation
- acetylation

FUS
TDP43
Nucleolin

https://www.phosphosite.org

NMR is cool & biophysical assays

Legend: PTM sites
- Phosphorylation
- Acetylation
- Ubiquitylation
- Other
Disordered regions play key roles in import & LLPS

LLPS-promoting regions

PTMs

cofactors

Fused-in-sarcoma (FUS)

Inclusions in ALS & FTD patients

Gomes et al., JBC (2019)
Zhang et al., Progr Neurobiol (2023)
Lenard et al., Cells (2022)
Zhou et al., Protein Sci (2021)
Zhang et al., Cell Rep Meth (2021)
Hutten et al., Cell Rep (2020)
Bourgeois et al., PNAS (2020)
Hofweber et al., Cell (2018)
Suárez-Calvet et al., Acta Neuropathologica (2016)
Dormann et al., EMBO J (2012)
Disordered regions play key roles in signaling

- Dynamic Protein complexes (Destruction Complex)
- Post-translational modifications (PTMs)
- Cellular transport
- Co-factors (β-catenin, ICAT)
- Diseases & ageing
- Transcription factor regulation (FOXO, FOXP, FOXM1, p53, Wnt)

**Scaffold proteins** (Axin-1, APC)

**Cytosol**

Alderson et al., JMRO (2022)
Spreitzer et al., CRISB (2022)
Usluer et al., IJMS (2021)
Bourgeois et al., Cell Rep (2021)
Richter et al., FEBS J (2021)
Göbl et al., Redox Biol (2020)
Merle et al., JMB (2019)
Hartlmüller et al., JBNMR (2019)
Bomblies et al., Plos One 12 (2017)
Anvarian et al., NSMB 23 (2016)
Baar et al., Cell 169 (2017)
Putker et al., Mol Cell 49 (2013)
Disordered regions play key roles in signaling

Diseases & ageing
Post-translational modifications (PTMs)

Benjamin Bourgeois
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Nucleus
Cytosol

Co-factors
RBPs
PTMs
Disease
mutations
Transcriptional condensates

TF1
TF2

Alderson et al., JMRO (2022)
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Usluer et al., IJMS (2021)
Bourgeois et al., Cell Rep (2021)
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Putker et al., Mol Cell 49 (2013)
FOXOs are master regulators

Aging, ROS-related diseases

Obesity, Diabetes

Inflammatory & immune disease

Defects in progenitor cells

Tumorigenesis, Infertility

Metabolism, Homeostasis

Cell-fate decisions

FOXO: Forkhead box O

ROS: Reactive Oxidative Species
The PI3K/PKB/FOXO module

PI3K: phosphatidylinositol 3-kinase
PTEN: phosphatase and tensin homologue on chromosome 10
PKB: protein kinase B
PDK1: phosphoinositide-dependent kinase-1

How do disordered regions of FOXOs act as regulatory hub?

Benjamin Bourgeois
FOXOs are challenging for structural biology

FOXO

Forkhead NLS

NES TA

PTM ‘code’

Phosphorylation
Acetylation
Ubiquitination
Methylation

PDB 3L2C

DNA binding

PONDR score

Residue number

Order

Residue

FOXO

237-KWSGGPC33NREAHIVWTTFRRSSNASVSTRRLSQL-274
FOXO1

294-KWAFSGSHSN6FDWWS7FRRTSSNAST18GLSP1-331
FOXO3

290-EPWSPFTH25DVLAA70FPSSTN52SVTGVQLSLP1-329

CR1 PH CR2 CR3
β-catenin binds FOXO4 disordered region

β-catenin binds FOXO4 disordered region
β-catenin binds FOXO4 disordered region

- **Polarization (A.U.)**
  - FOXO4\textsuperscript{CR3/PKB/AKT} $K_v = 80.4 \pm 12.4 \mu M$
  - FOXO4\textsuperscript{CR3} $K_v = 32.3 \pm 1.3 \mu M$

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Tianshu Gui and Boudewijn
β-catenin binds FOXO4 disordered region

- CR1 (≫ 100 μM): Overlaps with NLS and acetylation/ubiquitination sites
- CR2 (80 μM): Overlaps with methylation and phosphorylation sites
- CR3 (30 μM): Matches β-catenin binding consensus DXθXφX_{2-7}E

Acetylation  Phosphorylation  Ubiquitination  Methylation
FOXO4 is phosphorylated at the CR PKB/AKT

Cell lysate

Recombinant kinases
Phosphorylation blocks $\beta$-catenin binding

Tianshu Gui and Boudewijn Burgering
FOXO4 is auto-inhibited
FOXO4 is auto-inhibited
FOXO4 is auto-inhibited

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<tr>
<td>FOXO4&lt;sup&gt;FH&lt;/sup&gt;</td>
<td>DNA</td>
<td>200 ± 17 nM</td>
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<tr>
<td>FOXO4&lt;sup&gt;FH-CR3&lt;/sup&gt;</td>
<td>DNA</td>
<td>2090 ± 382 nM</td>
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<tr>
<td>FOXO4&lt;sup&gt;FH-CR3&lt;/sup&gt; + β-catenin</td>
<td>DNA</td>
<td>277 ± 8 nM</td>
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PDB 3L2C
In cell FRET confirms FOXO4 conformational change.
Auto-inhibition is conserved among FOXOs

ICAT regulates FOXO4/TCF/LEF binding to β-catenin

Tianshu Gui and Boudewijn Burgering
Klaus Richter
ICAT regulates FOXO4/TCF/LEF binding to β-catenin

**Analytical Ultracentrifugation**

FOXO4 complexes

- + β-catenin/ICAT
- + β-catenin

**Molecular mass (collaboration Klaus Richter, TUM)**

PDB 1LUJ, 1G3J
ICAT regulates FOXO4/TCF/LEF signaling
\(\beta\)-catenin competes with FOXO auto-inhibition
β-catenin competes with FOXO auto-inhibition
$\beta$-catenin - FOXO interaction network

ICAT blocks TCF/LEF and restores binding

TCF/LEF, Axin-1, APC, cadherin inhibit binding

PTMs inhibit binding (P, ArgMet)
Targeting disordered regions of transcription factors

Emil Spreitzer
Benjamin Bourgeois

\[ K_D \approx 1.8 \pm 0.2 \, \mu\text{M} \]

K\textsubscript{D}: 1.8 ± 0.2 µM

Collaboration
Peter de Keizer

WO 2021/165538 Improved anti-senescence compounds and uses thereof
DeKeizer, Campisi, Hoeijmakers and Madl labs – Cell 169 (2017) 132
Here we are and there we go

The interaction network between FOXO4 and β-catenin was deciphered.

FOXO4 auto-inhibition interferes with DNA binding and is counter-acted by β-catenin.

FOXO4 exists in multiple conformations regulated by phosphorylation and co-factors.

Challenging disordered p53 transcription factor can be targeted with peptides.

Targeting p53 eliminates senescent cells in vitro and in vivo.

Discoveries and new concepts might enable targeting of other promising disordered proteins in ageing.
Thank you!

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