Deciphering the Role of Posttranslational Modifications on the DNA-Binding Activity of Max Transcription Factor Using Chemical Protein Synthesis

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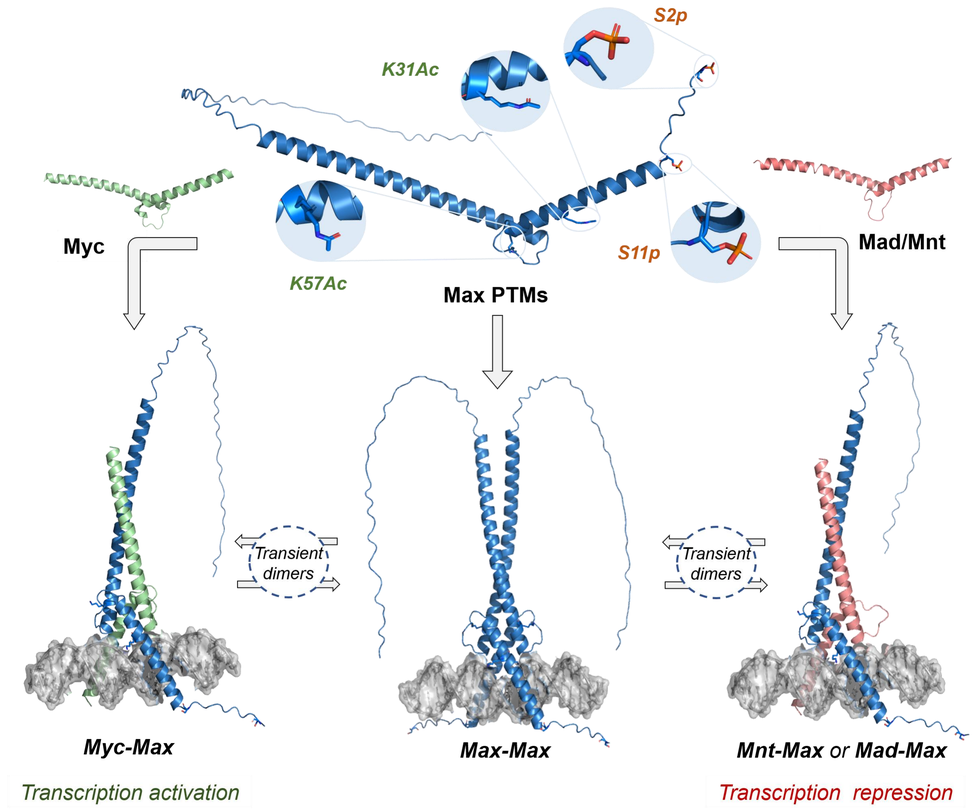
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Abstract

Transcription factors (TFs) are proteins that regulate gene transcription through sequence-specific DNA binding, and their activities are tightly controlled by dynamic post-translational modifications (PTMs). The Myc–Max–Mad TF network plays a pivotal role in regulating the expression of approximately 15% of the human genome, and its dysregulation is implicated in nearly 70% of human cancers. Within this network, the selective dimerization of Max with its binding partners, including Myc, Mad, and Mnt, is central to transcriptional regulation. Max activity is regulated by PTMs, with Ser-phosphorylation and Lys-acetylation considered key regulatory mechanisms. However, the precise molecular mechanisms by which these PTMs regulate Max oligomerization and DNA binding remain elusive. In this study, we developed a practical synthetic strategy for the preparation of homogeneous full-length Max, enabling the systematic investigation of site-specific PTM effects on its DNA-binding activity. Using this approach, we generated a focused library of Max variants incorporating defined phosphorylation, acetylation, and fluorescent modifications. Comprehensive DNA-binding analyses revealed that both phosphorylation and acetylation significantly inhibit Max DNA binding activity. Notably, our results further show that PTMs, e.g. Lys-acetylation, can alter the binding specificities of Max towards certain sequences flanking its consensus binding sites. Furthermore, the total synthesis of site-specifically labelled Myc, Mnt, and Mad TF analogs enabled the combinatorial assembly of 24 distinct Max heterodimeric complexes, allowing systematic characterization of dimerization and DNA-binding properties across the network. Our work provides insights into the hidden molecular code of PTM-dependent regulation of TF-DNA interactions, paving the way to interpret gene expression regulation programs and engineering bioactive TFs.

Regulation of the Myc–Max–Mad Transcription Factor Network by Posttranslational Modifications

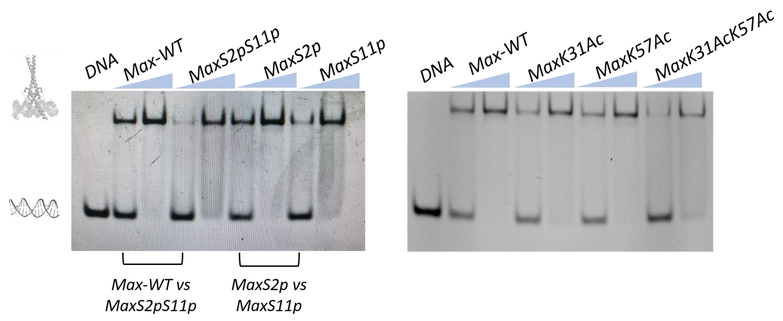


- Controls the expression of ≈15% of the entire genome
- Implicated in over 70% of all human cancers
- Challenging drug target

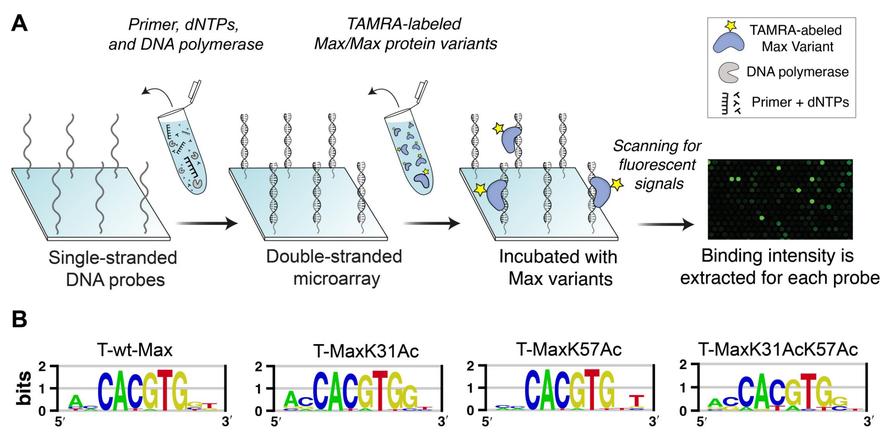
- Max is Essential for heterodimerization & activity
- Endogenous inhibitor for Myc (*homodimerization*)
- Potential synthetic regulator

DNA Binding Activity of Site-specifically Modified Max Analogs

Electrophoretic Mobility-Shift Assay (EMSA)



In Vitro High-Throughput Analysis Using a Protein Binding Microarray



Biolayer Interferometry Assay (BLI)

Protein	K_D (E-box)
Max-WT	15.3 ± 0.3 nM
MaxS2pS11p	54.4 ± 0.6 nM
MaxS2p	23.9 ± 0.4 nM
MaxS11p	41.9 ± 1.1 nM
MaxK31AcK57Ac	294.3 ± 3.0 nM
MaxK31Ac	134.0 ± 13.9 nM
MaxK57Ac	51.8 ± 6.8 nM
MaxS2pS11pK31AcK57Ac	1100.0 ± 30.0 nM

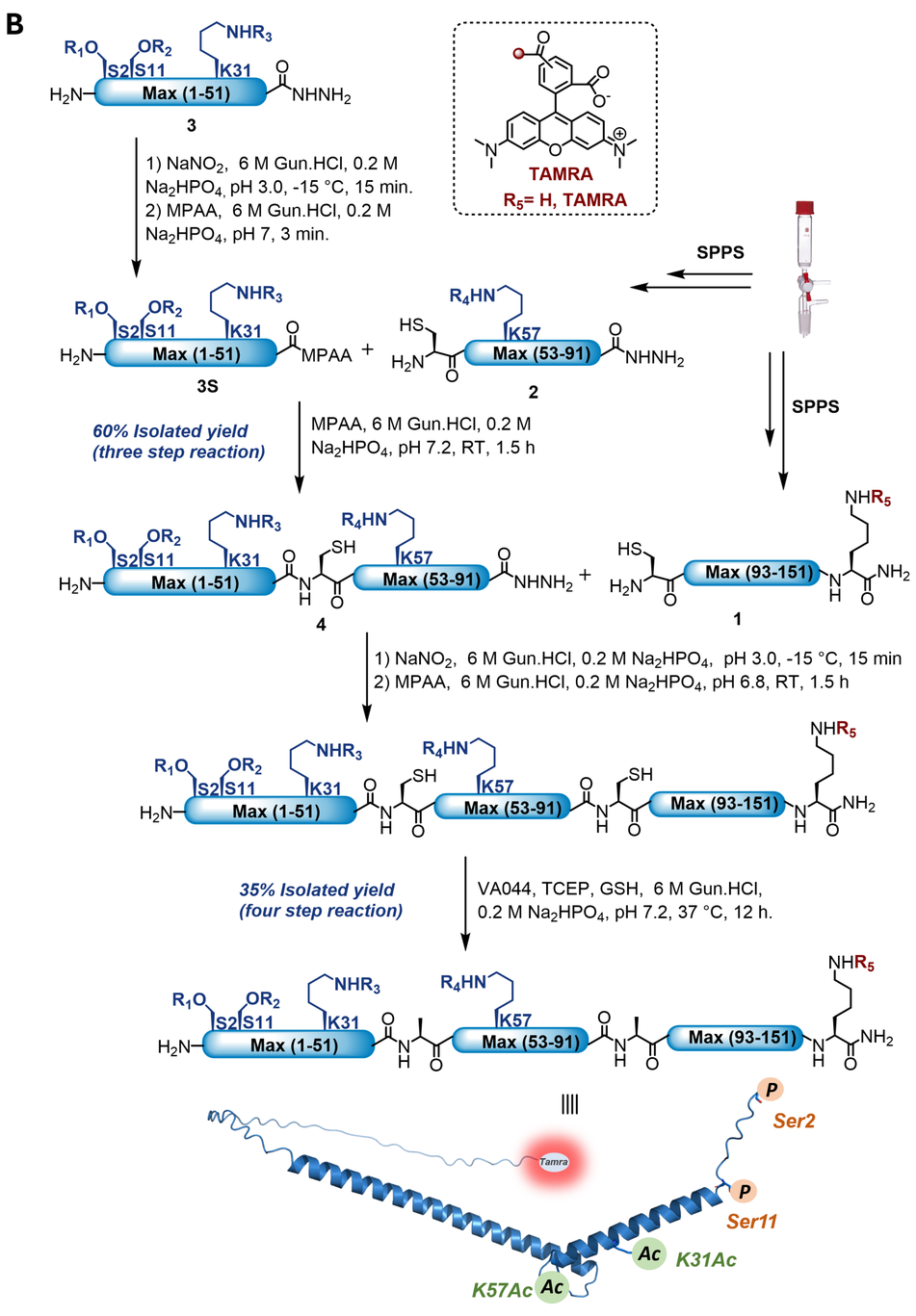
- ***Ser-phosphorylation and Lys-acetylation significantly inhibit the DNA binding activity of Max TF***
- ***PTM marks do not significantly impact Max's binding specificity to the core E-box sequence (CACGTG)***
- ***Doubly acetylated variant displays a robust preference for DNA sequences with 'GG' at positions +3 and +4.***

Design and Chemical Synthesis of Site-specifically Modified Max Transcription Factor Analogs

A

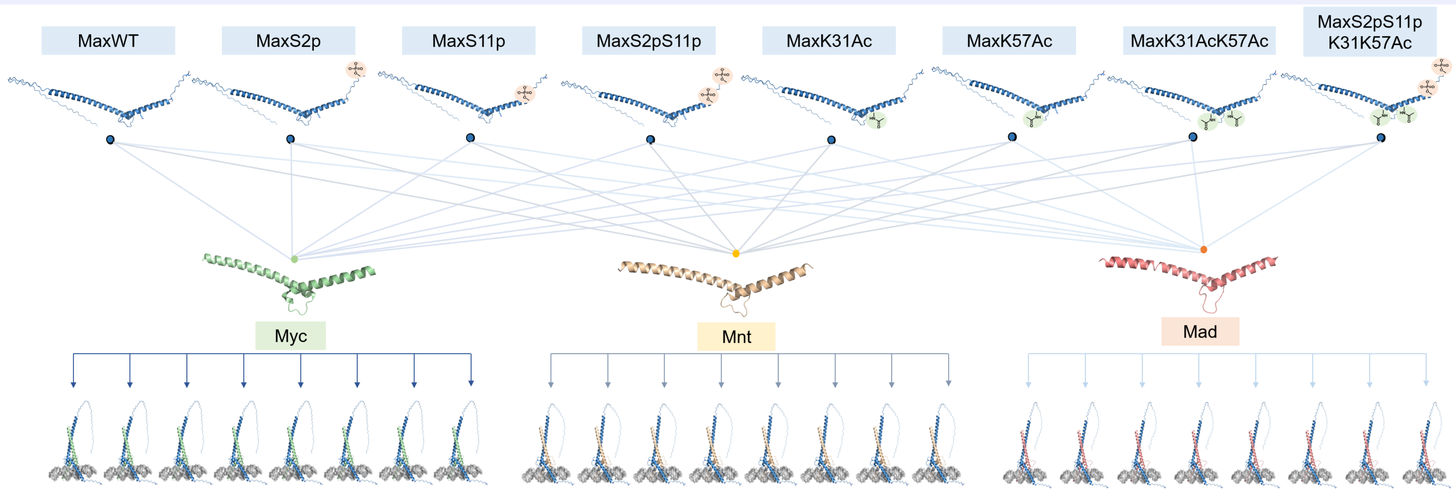
MS[2]DNDDIEVES[11]DADKRAHHNALERKRRDHIK[31]DSFHSLRDSVPSLQGEKASRA
QILDK[57]ATEYIQYMRRKNHTHQQDIDDLKRQNALLEQQVRALEKARSSAQLQTNY
PSSDNSLYTNAKGSTISAFDGGSDSSSESEPEEP QSRKKLRMEAS

B



- ***Developed a practical synthetic strategy to prepare homogeneous full-length Max transcription factor for the first time and generated a focused library of 15 distinct analogs with defined modification patterns, including site-specific phosphorylation, acetylation, and fluorescent tagging.***

The Myc–Max–Mad Oligomerizations and DNA Binding Activity are Modulated by Both Specific PTM Pattern and Transcription Factor Partner

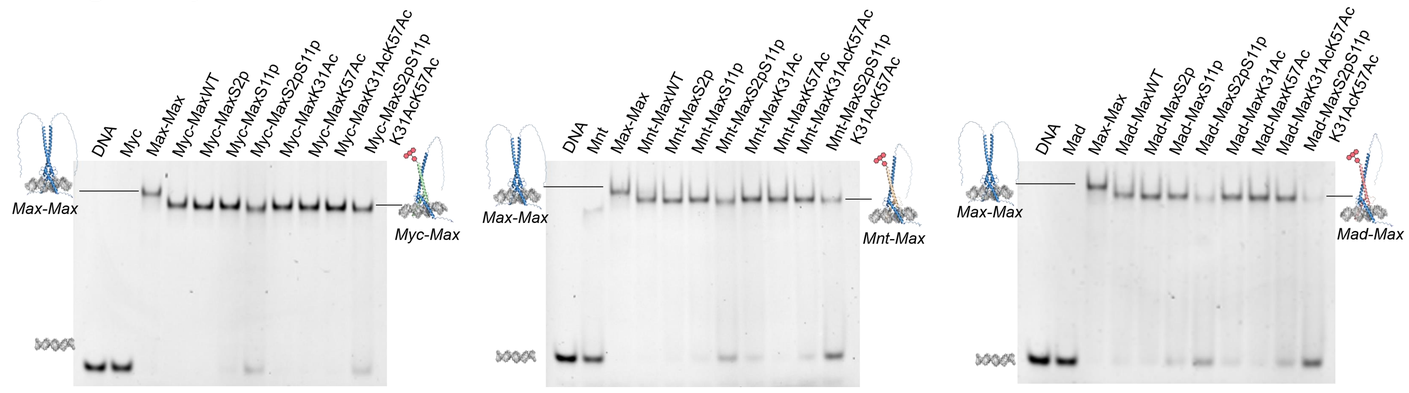


Myc-Max-Mad Interactions via BLI Assay

Myc-Max interaction	K_D (nM)	Mnt-Max interaction	K_D (nM)	Mad-Max interaction	K_D (nM)
Myc-MaxWT	74.6	Mnt-MaxWT	214.8	Mad-MaxWT	208.7
Myc-MaxS2pS11p	58.8	Mnt-MaxS2pS11p	142.4	Mad-MaxS2pS11p	143.1
Myc-MaxK31AcK57Ac	79.1	Mnt-MaxK31AcK57Ac	139.7	Mad-MaxK31AcK57Ac	196.0
Myc-MaxS2pS11pK31AcK57Ac	74.3	Mnt-MaxS2pS11pK31AcK57Ac	73.6	Mad-MaxS2pS11pK31AcK57Ac	95.1

- ***Ser-phosphorylation in combination with Lys-acetylation selectively enhances Max heterodimerization with Mnt and Mad, but not with Myc***

DNA Binding Activity of Max Heterodimers via EMSA



- ***PTMs markedly reduce DNA binding in Mnt–Max and Mad–Max complexes, whereas they are largely tolerated in Myc–Max complexes***
- ***Our work provides insight into the molecular code of PTM-TFs and DNA interactions, paving the way to interpret gene expression regulation programs***

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