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Re-examination of DAX-1 (NR0B1) RNA binding using public proteome-wide datasets

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Abstract

Background. In 2000, Lalli et al. (Mol Cell Biol 20:4910–4921) reported that the orphan nuclear receptor DAX-1 (NR0B1) binds RNA directly, co-purifies with poly(A)+ RNA after UV crosslinking, and associates with polyribosomes in adrenocortical and Leydig cells. The author of the present study is a co-author of that report. Proteome-wide RNA-binding protein (RBP) datasets published since then allow this claim to be re-examined independently.

Methods. I queried the RBP2GO 2.0 database and re-analysed five public mass-spectrometry datasets: nuclear RBR-ID in mouse embryonic stem cells (mESCs; He et al. 2016), mESC oligo(dT) interactome capture (Kwon et al. 2013), RNA interactome capture of mouse male germ cells (Li et al. 2024; PRIDE PXD043074), a deep mESC whole-cell proteome (PRIDE PXD016729) and the mESC ribosome interactome (Simsek et al. 2017). Each dataset was judged by the significance criteria defined in its original publication.

Results. NR0B1 was listed in 3 of 62 human RBP2GO datasets, none of them experimental. Dax1 was quantified in all 30 mESC whole-proteome samples (8 unique peptides, 33.1% coverage). In mESC nuclei, 19 Dax1 peptides spanning both the N-terminal repeats and the ligand-binding-like domain were quantified by RBR-ID; none met the primary (log2 fold change < 0, Student's t-test $p < 0.05$) or extended ($p < 0.1$) criterion. In the mESC ribo-interactome, Dax1 gave 2 spectra in 1 of 6 bait replicates (SAINT 0.233, FDR 0.247), below the published cut-off (SAINT ⩾ 0.56, FDR ⩽ 0.08, ⩾ 4-fold enrichment). Dax1 was not identified in either oligo(dT) capture dataset, but neither were other pluripotency transcription factors that are readily detected in the whole-cell proteome.

Conclusions. Public proteome-wide data from mESCs do not support RNA binding or ribosome association of Dax1, although Dax1 is abundant enough to be quantified. No comparable data yet exist for steroidogenic cells, the cell types studied in 2000, so the original claim remains untested there.

Introduction

DAX-1 (NR0B1) is an atypical nuclear receptor that lacks a zinc-finger DNA-binding domain. Its N terminus consists of three repeats of about 70 residues, and its C terminus resembles a nuclear receptor ligand-binding domain (LBD). Loss-of-function mutations cause X-linked adrenal hypoplasia congenita with hypogonadotropic hypogonadism (AHC-HHG), and duplication of the locus causes dosage-sensitive sex reversal.

Lalli et al. (2000) proposed that DAX-1 is a shuttling RNA-binding protein. Their evidence was: (i) nuclear and cytoplasmic localisation in H295R adrenocortical and MA-10 Leydig cells; (ii) co-sedimentation with polyribosomes, shifted by EDTA and RNase; (iii) co-elution with poly(A)+ RNA on oligo(dT) after in vivo UV crosslinking; (iv) binding to ribohomopolymers in the order poly(U) > poly(G) >> poly(A) > poly(C), mediated cooperatively by the N-terminal repeats and the LBD-like domain; and (v) reduced homopolymer binding by AHC mutants (R267P, ΔV269, N440I and a 1–451 truncation). The report has since been cited as evidence that DAX-1 is an RBP, for example in the Atlas of Genetics and Cytogenetics in Oncology and Haematology.

Since 2012, proteome-wide methods such as interactome capture, RBR-ID and ribosome affinity purification have catalogued RBPs in many cell types. These datasets were not designed to test DAX-1, but several were generated in mESCs, where Dax1 is expressed as part of the pluripotency network. Here I ask whether these public data reproduce claims (ii) to (iv) and, where possible, check that Dax1 protein was present at detectable levels.

Methods

All data were public and accessed on 13 November 2026. No new experiments were performed. For every dataset I applied the significance criteria stated by its original authors and did not re-derive statistics.

Dataset	System	Measurement	Source	Criterion applied
RBP2GO 2.0	62 human, 18 mouse datasets	Curated listing of proteome-wide RBP studies	rbp2gov2.dkfz.de	Listing count; study type

Dataset	System	Measurement	Source	Criterion applied
RBR-ID (He et al. 2016)	mESC nuclei	4SU photocrosslinking, peptide-level depletion	Bonasio Lab RBR-ID browser v0.3	Primary: log2FC < 0 and Student's t-test $p < 0.05$; extended: $p < 0.1$
Kwon et al. 2013	mESC	Oligo(dT) capture, crosslinked vs non-crosslinked, isotope labelling, 3 replicates	Supplementary Data Set 1	Listed in Table S2a (FDR < 1%) or identified in Table S2b
Li et al. 2024	Spermatogonia, pachytene spermatocytes, round spermatids	Oligo(dT) capture, UV vs no-UV	PRIDE PXD043074, MaxQuant proteinGroups.txt	Identified in any sample
Genolet et al. 2021	mESC line 1.8, 10 genotypes × 3 replicates	Whole-cell proteome, SCX fractionation, label-free	PRIDE PXD016729, proteinGroups.txt	Identified and LFQ intensity > 0
Simsek et al. 2017	mESC	FLAG-eL36 / FLAG-eS17 ribosome IP vs FLAG-GFP, 3 replicates each	Table S1 and S3	SAINT ⩾ 0.56, FDR ⩽ 0.08 and ⩾ 4-fold enrichment

RBP2GO was searched for NR0B1/Nr0b1 and, for comparison, NR5A1/Nr5a1 and splicing factor 1 (SF1; UniProt Q15637, Q64213). For RBR-ID, all Dax1 peptides (UniProt entry Q53ZY9 as mapped by the browser) were exported with log2FC, p value and RBR score, where score = $-\log2FC \times (\log10\ p)^2$. Hnrnpa1, Pou5f1, Nr5a2, Esrrb and Sox2 were retrieved the same way. MaxQuant tables were obtained from PRIDE by HTTP range requests and searched by gene name and by UniProt accession (Q61066). Peptide positions reported by the RBR-ID browser have not yet been re-mapped to the canonical mouse sequence.

Results

No experimental proteome-wide study lists DAX-1 as an RBP

In RBP2GO 2.0, human NR0B1 was listed in 3 of 62 datasets (RBP2GO score 3.5). The three were GO annotation (GO:0003723), a sequence-based prediction (TriPepSVM) and a literature review (Gerstberger et al. 2014). Mouse Nr0b1 was listed in 1 of 18 datasets, GO annotation only. Neither entry had an RNA-binding peptide. The mouse datasets that did not list Nr0b1 include four from mESCs and two from male germ cells.

Dax1 is abundant enough to be quantified in mESCs

In the mESC whole-cell proteome (PXD016729), Dax1 (Q61066) was identified with 8 unique peptides, 33.1% sequence coverage, 117 MS/MS spectra and MaxQuant Q-value 0. LFQ intensity was non-zero in all 30 samples (median 4.85×10^7; range $3.3–8.2 \times 10^7$). In a subset of 921 protein groups retrieved from the same table, this median ranked 382nd, above Klf4 (2.4×10^7) and Tfcp2l1 (2.8×10^7). Pou5f1, Esrrb, Sox2, Tbx3 and Dppa3 were each quantified in 29 or 30 of 30 samples.

Nuclear RBR-ID: no Dax1 peptide meets either criterion

RBR-ID quantified 19 Dax1 peptides: 8 in the N-terminal repeats (residues 2–162) and 11 in the LBD-like domain (residues 235–452). Five had log2FC < 0, with p values of 0.16 to 0.91. The highest RBR score was 0.156 (residues 252–266; log2FC −1.15, p = 0.43). The only peptide with $p < 0.05$ (residues 428–439) changed in the opposite direction (log2FC +0.30). Dax1 therefore falls in neither the primary nor the extended RBR-ID list.

Protein	Peptides quantified	Best-scoring peptide (log2FC, p)	Highest RBR score	Outcome
Hnrnpa1	43	residues 44–49 (−1.38, 0.044)	8.76	Primary hit
Pou5f1	20	residues 138–144 (−3.77, 0.039)	7.53	Primary hit
Nr5a2	13	residues 454–460 (−12.3, 0.085)	14.1	Extended list
Esrrb	33	residues 189–206 (−12.9, 0.24)	5.07	Not significant
Sox2	12	residues 45–55 (−1.06, 0.12)	0.88	Not significant

Protein	Peptides quantified	Best-scoring peptide (log2FC, p)	Highest RBR score	Outcome
Dax1	**19**	**residues 252–266 (−1.15, 0.43)**	**0.156**	**Not significant**

Oligo(dT) capture: Dax1 is absent, as are other transcription factors

Kwon et al. (2013) identified 1,236 proteins in oligo(dT) eluates and listed 555 as mRNA-bound. Dax1 was not among the 1,236. Pou5f1, Esrrb, Nanog and Nr5a2 were also absent. Sox2 appeared only in a protein group shared with Sox1, Sox3 and Sox21, without quantification. Positive controls were enriched, for example Hnrnpa1 (mean log2FC +4.75) and Lin28a (+5.39).

In male germ cells (PXD043074), 2,066 (spermatogonia), 2,540 (pachytene spermatocytes) and 1,837 (round spermatids) protein groups were identified. Dax1 was absent at all three stages, as were Nr5a1, Nr5a2, Pou5f1, Sox2 and Esrrb. Hnrnpa1 and Ddx4 were enriched in UV-crosslinked samples. Neither study measured an input proteome.

Ribosome interactome: Dax1 is below the published cut-off

In Simsek et al. (2017), Dax1 gave 2 spectra in 1 of 3 eL36 replicates, none in the eS17 replicates and none in GFP controls. Its SAINT score was 0.233, minimum FDR 0.247 and log2 maximum fold change 1. The published cut-off was SAINT ⩾ 0.56, FDR ⩽ 0.08 and ⩾ 4-fold enrichment. Lin28a met all three (SAINT 0.97, FDR 0.0017, about 6-fold). Dax1 was absent from the TMT table of RNase- and puromycin-treated pulldowns.

Summary

Dataset	System	Dax1 detected	RNA or ribosome association
Whole proteome (PXD016729)	mESC	Yes, 30/30 samples	Not applicable
RBR-ID	mESC nuclei	Yes, 19 peptides	Not significant
Ribo-interactome	mESC	2 spectra, 1/6 replicates	Below cut-off
Oligo(dT) capture (Kwon 2013)	mESC	No	Not assessable; other TFs also absent

Dataset	System	Dax1 detected	RNA or ribosome association
Oligo(dT) capture (Li 2024)	Male germ cells	No	Not assessable; expression uncertain

Discussion

In mESCs, where Dax1 is readily quantified, three independent proteome-wide assays gave no evidence that it binds RNA or ribosomes at the thresholds set by their authors. RBR-ID is the most informative of these. It measured 19 Dax1 peptides covering both domains that Lalli et al. (2000) implicated in RNA binding, and none was depleted by 4SU-dependent crosslinking. The same assay detected Pou5f1 and Hnrnpa1, so its negative result for Dax1 is not explained by a general insensitivity to transcription factors.

The oligo(dT) datasets are less informative. Dax1 was absent from mESC eluates, but so were Pou5f1, Esrrb and Nanog, which the whole-cell proteome detects. Absence from these eluates is therefore common to pluripotency transcription factors and not specific to Dax1. In male germ cells, Dax1 expression is itself uncertain, because testicular DAX-1 is reported mainly in Leydig and Sertoli cells.

Limitations

- **Cell type.** All informative data come from mESCs. The original study used human H295R adrenocortical and mouse MA-10 Leydig cells, and no proteome-wide RBP or ribosome dataset exists for steroidogenic cells. The present results cannot exclude cell-type-specific RNA binding.
- **Compartment.** RBR-ID was performed on nuclei only. The original claim of polyribosome association concerns cytoplasmic DAX-1, which RBR-ID does not test.
- **Assay scope.** Ribosome affinity purification captures ribosomes in general, not the sucrose-gradient polysome fraction used in 2000.
- **Statistical power.** Each RBR-ID comparison used three replicates and a Student's t-test. Non-significance does not prove absence of binding, and 4SU crosslinking detects only direct contacts.
- **Cross-study comparison.** Dax1 abundance was established in a different mESC line and laboratory from the oligo(dT) and ribosome datasets.
- **Residue mapping.** RBR-ID peptide positions follow the browser's UniProt mapping and should be checked against the canonical sequence before the AHC mutation sites are interpreted.

A note on SF-1 in RBP2GO

Human NR5A1 (steroidogenic factor 1) was listed in 9 of 62 RBP2GO datasets, including interactome capture in HeLa, HEK293, K562 and HuH-7 cells and RNAcompete. NR5A1 is not expressed in these cell lines, whereas splicing factor 1 (SF1; Q15637) is listed in 46 datasets. This pattern suggests that the shared alias "SF1" was mapped to NR5A1. This has not yet been confirmed against the UniProt accessions in the original supplementary tables.

Conclusion

Public proteome-wide data do not reproduce RNA binding or ribosome association of Dax1 in mESCs. Whether DAX-1 binds RNA in steroidogenic cells, as reported in 2000, remains open. The question could be settled by interactome capture or by UV crosslinking with oligo(dT) pulldown in H295R cells, using established RBPs as positive controls and RNase-treated samples as negative controls.

Declarations

Competing interests. The author is a co-author of Lalli et al. (2000), the study re-examined here. No other competing interests.

Funding. This work received no specific funding.

Ethics statement. No new experiments on humans or animals were performed. Only publicly available, de-identified data were analysed, so ethics approval and consent were not required.

Data availability. All data are public: RBP2GO 2.0 (rbp2gov2.dkfz.de), the Bonasio Lab RBR-ID browser (rbrid.bonasiolab.org), PRIDE PXD016729 and PXD043074, and the supplementary tables of Kwon et al. (2013) and Simsek et al. (2017). Extracted values are reported in full in the tables above.

Use of AI tools. Data retrieval, table extraction and drafting were assisted by Claude (Anthropic). The author checked all values and takes responsibility for the content.

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