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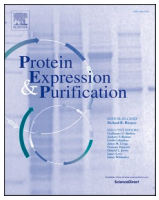

Comparative analysis of protein expression systems and PTM landscape in the study of transcription factor ELK-1

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ABSTRACT

Post-translational modifications (PTMs) are important for protein folding and activity, and the ability to recreate physiologically relevant PTM profiles on recombinantly-expressed proteins is vital for meaningful functional analysis. The ETS transcription factor ELK-1 serves as a paradigm for cellular responses to mitogens and can synergise with androgen receptor to promote prostate cancer progression, although *in vitro* protein function analyses to date have largely overlooked its complex PTM landscapes. We expressed and purified human ELK-1 using mammalian (HEK293T), insect (Sf9) and bacterial (*E. coli*) systems in parallel and compared PTMs imparted upon purified proteins, along with their performance in DNA and protein interaction assays. Phosphorylation of ELK-1 within its transactivation domain, known to promote DNA binding, was most apparent in protein isolated from human cells and accordingly conferred the strongest DNA binding *in vitro*, while protein expressed in insect cells bound most efficiently to the androgen receptor. We observed lysine acetylation, a hitherto unreported PTM of ELK-1, which appeared highest in insect cell-derived ELK-1 but was also present in HEK293T-derived ELK-1. Acetylation of ELK-1 was enhanced in HEK293T cells following starvation and mitogen stimulation, and modified lysines showed overlap with previously identified regulatory SUMOylation and ubiquitination sites. Our data demonstrate that the choice of recombinant expression system can be tailored to suit biochemical application rather than to maximise soluble protein production and suggest the potential for crosstalk and antagonism between different PTMs of ELK-1.

1. Introduction

Heterologous expression and purification of proteins is key to experimentally studying their structure and function and, as such, a wide range of prokaryotic and eukaryotic expression systems have been developed and refined over many years. Detailed biochemical analyses of purified human health-relevant proteins (such as oncoproteins) produced using various expression systems can provide insight into the molecular mechanisms underpinning disease pathogenesis [1–3]. Commonly, optimised strains of *Escherichia coli* (*E. coli*) are transformed with a plasmid containing the protein of interest, grown to log phase, and induced, before isolation of the protein of interest through purification methodologies. Benefits of *E. coli* use include its fast growth rate under optimal conditions and potential for large protein output at low cost. However, protein functionality is not guaranteed, as some proteins will fail to fold correctly in a bacterial expression system and/or be sequestered in inclusion bodies, hampering viable purification [4]. One important factor in this regard is appropriate regulatory post-translation modification (PTM) of the protein of interest, such as phosphorylation, glycosylation and acetylation, which may be essential for optimal protein function and for which *E. coli* systems are generally less than adequate in providing [5].

To this end, eukaryotic expression systems may be more appropriate. These include expression-vector transformation of yeast (e.g. *Saccharomyces cerevisiae*, *Pichia pastoris*), infection of lepidopteran insect cells (e.g. *Spodoptera frugiperda Sf*9/*Sf*21, *Trichoplusia ni*) with recombinant baculovirus vectors, and transient transfection or stable cell line generation using immortalised mammalian cells (e.g. CHO, HEK293 [6,7]). Each system has advantages and disadvantages depending on the specific requirements of the target protein, as well as differences in cost and scalability. For example, expression of human protein in mammalian expression systems reaps the benefits of a cognate repertoire of PTM enzymes, with the downside of more demanding culture conditions and far slower growth and yield compared with microbial-based methods

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[7].

The E-twenty-six/E26 (ETS) family member and ternary complex factor (TCF) ELK-1 has been extensively studied as a mitogen-responsive transcription factor activated upon phosphorylation by extracellular signal-regulated kinases (ERKs) within its C-terminal transactivation domain (TAD, Fig. 1a) [8–10]. The conserved winged helix-turn-helix DNA-binding domain that defines the ETS family forms contacts with purine-rich DNA sequences with a GGA core and represents one of the two regions of defined structure in TCFs, alongside the B domain [11–13]. In response to growth factor signalling, ELK-1 forms a ternary complex through the B domain with a dimer of Serum Response Factor (SRF) at Serum Response Elements (SREs) in target genes such as *CFOS* and *EGR1*, promoting their transcription and driving cell-cycle entry and proliferation [14–17]. Transcriptional activity can also be suppressed by SUMOylation in a repression domain (R) and mono-ubiquitination in the ETS domain [18–20]. ELK-1 has been identified as a possible therapeutic target in prostate cancer (PC) due to interactions with the androgen receptor (AR), in depression, and Huntington's disease [21–25]. Therefore, expression and purification of functional ELK-1 is of importance to allow detailed studies of relevant disease-state interactors, such as AR in the context of PC.

In this study, we have expressed full-length human ELK-1 using bacterial, insect and mammalian systems in parallel and carried out protein purification to permit comparative analysis of PTM and protein function (DNA and protein binding). Differential PTM profiles were observed with proteins resulting from each methodology, with optimal ELK-1-DNA and ELK-1-AR interactions observed with human and insect cell-derived ELK-1 protein, respectively. By stimulating mammalian cells, we have also identified sites of lysine acetylation in ELK-1 that overlap with sites of other PTMs, implying competition within the ELK-1 PTM landscape. Our work highlights the need to match the target to the expression system, and to the downstream experimental or functional applications for the desired protein.

2. Materials and methods

2.1. Plasmids and antibodies

Vectors encoding ELK-1-His (human, UniProt ID P19419) for expression in human (pCMV5), insect (pFastBac1) and bacterial cells (pQE60) were available from previous studies [10,26]. Human AR-N-His (1–560) cDNA was sub-cloned into pGEX2T for bacterial expression with an N-terminal glutathione S-transferase (GST)-tag. Full-length human AR cDNA inserted into polylinker of CMV-FLAG (pCMV5) was used for expression in human cells. Antibodies used were as follows: ELK-1 rabbit monoclonal (Abcam ab32106), phospho-ELK-1 (S383) rabbit polyclonal (Cell Signalling Technology #9181), phospho-tyrosine mouse monoclonal (Merck Millipore 4G10), acetyl-lysine rabbit polyclonal (Upstate Biotechnology), GST-HRP conjugate (Amersham RPN1236), AR rabbit polyclonal (GeneTex GTX100056), phospho-ERK1/2 (T202/Y204) rabbit monoclonal (Cell Signalling Technology #4376), ERK2 mouse monoclonal (Santa Cruz Biotechnology sc-1647).

2.2. Cell culture

HEK293T cells were cultured at 37 °C (7.5% CO_2) in low glucose DMEM supplemented with 10% foetal calf serum (FCS), 100 U/ml penicillin, 100 μg/ml streptomycin and 2 mM L-glutamine. Where

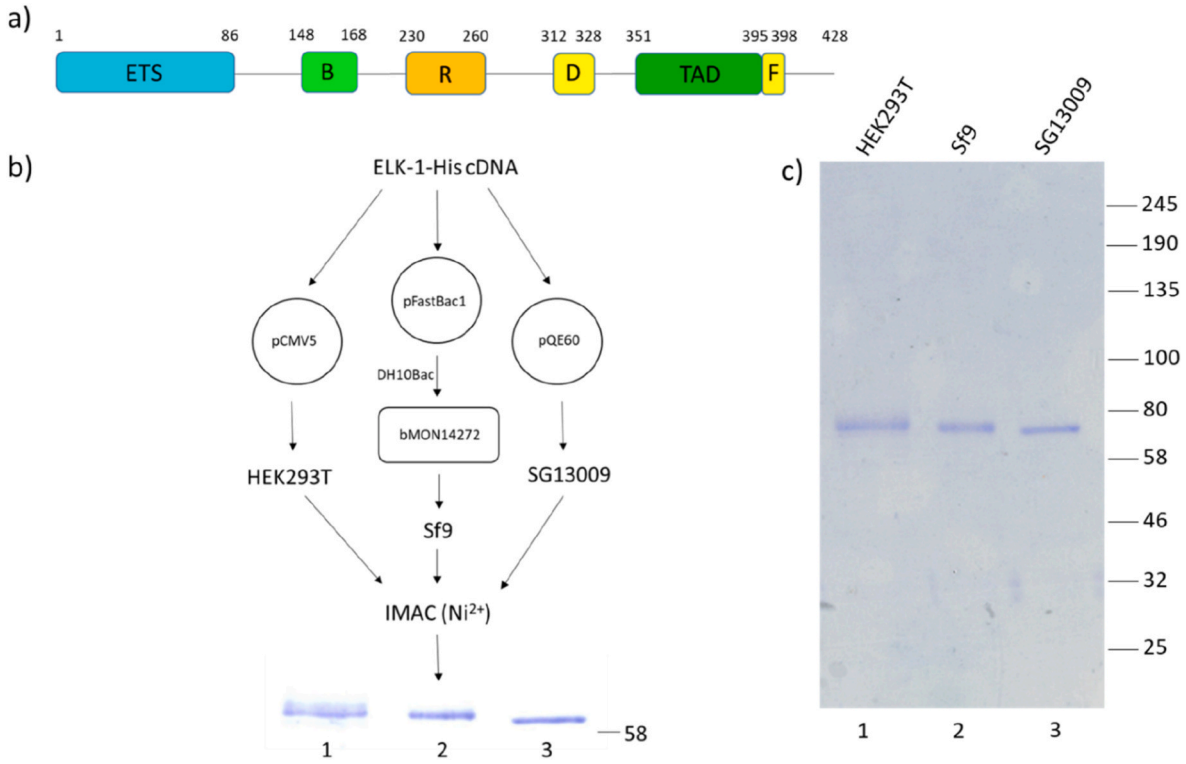

Fig. 1. a) Schematic of human ELK-1 illustrating functional domains. ETS – DNA-binding, B – SRF interaction, R – SUMOylation and repression, D/F – MAP kinase docking, TAD – transactivation. **b)** Human ELK-1-His cDNA was cloned into appropriate plasmids and expressed as follows: *Mammalian* – pCMV5-ELK-1 was transiently transfected into HEK293T cells. *Insect* - pFastBac1-ELK-1 was transformed into DH10Bac *E. coli* allowing transposition between pFastBac1 and shuttle vector (bacmid) bMON14272 through transposase activity conferred from helper plasmid pMON7124. Recombinant bacmid DNA was transfected into Sf9 cells, with virus harvested and used to infect Sf9 cells for protein expression. *Bacterial* – pQE60-ELK-1 was transformed into SG13009 *E. coli* (harbouring pREP4 plasmid for lacI expression) and used to generate liquid cultures, which were grown to log phase and induced with IPTG. All ELK-1-His proteins were subsequently purified by IMAC and resolved by SDS-PAGE. **c)** Equal loads of ELK-1 proteins (1.5 μg) resolved by SDS-PAGE on a gradient (5–20%) gel (MW markers indicated right of gel image, kDa).

indicated, cells were serum-starved in growth medium supplemented with 0.5% FCS for 24 h and stimulated with 15% FCS and 100 ng/ml 12-*O*-tetradecanoylphorbol-13-acetate (TPA) for 30 min. Sf9 cells were maintained at 27 °C in Insect-Xpress medium supplemented with 10% FCS, 200 U/ml penicillin and 200 μg/ml streptomycin.

2.3. Protein expression and purification

Mammalian - HEK293T cells were transfected with pCMV5-ELK-1-His *via* calcium phosphate precipitation and harvested after 48 h. *Insect* – pFastBac1-ELK-1-His was transformed into DH10Bac *E. coli* on LB agar containing 30 μg/ml kanamycin, 7 μg/ml gentamicin, 10 μg/ml tetracycline, 100 μg/ml X-gal and 40 μg/ml IPTG with white colonies indicative of transposition into baculoviral genome selected. Recombinant bacmid was transfected into Sf9 cells with Escort IV reagent, with supernatant harvested after 7 days. Baculovirus-infected insect cell (BIIC) stocks were generated by infection of Sf9 cells with supernatant and harvested when mean cell diameter increased by ~1.5 μm. BIIC stocks were used to infect Sf9 cells and harvested after 48 h. *Bacterial* – pQE60-ELK-1-His was transformed into SG13009 *E. coli* cells on LB agar containing 25 μg/ml kanamycin and 100 μg/ml ampicillin. LB cultures were grown at 37 °C and cells were induced at OD^{600}~0.6 with 1 mM IPTG for 4 h at 30 °C before harvesting.

Protein purification was the same across all ELK-1-His preparations, with pellets lysed in phosphate buffer (50 mM NaH_2PO_4 pH 7.0, 500 mM NaCl, 300 mM KCl, 20 mM imidazole, 1% v/v NP-40), sonicated 8 × 10 s pulses (amplitude 25 Hz) followed by centrifugation (14,000 rpm, 4 °C, 15 min). Supernatant was incubated with 1 ml nickel-NTA agarose beads (Qiagen) for 2 h (4 °C), washed with phosphate buffer and eluted with imidazole (50–500 mM gradient). Purified proteins in the 200–500 mM imidazole fractions were dialysed against HEPES-buffered saline (HBS-10 mM HEPES pH 7.4, 150 mM NaCl, 1 mM DTT).

2.4. Protein mass spectrometry

Coomassie-stained protein bands from SDS-PAGE (1.5 μg) were excised, reduced, alkylated, digested with trypsin and submitted to tandem mass spectrometry (MS/MS) on an LTQ Orbitrap spectrometer with nanoflow liquid chromatography. Peptides were identified in data-dependent mode, using Mascot (2.7.0) to search the human UniProt (2018) and cRAP (2019) databases (93734 proteins, 2 max missed cleavage sites, precursor ion mass tolerance 20 PPM, fragment ion mass tolerance 0.100 Da) and validated with Scaffold (4.11.1). Total Spectrum Counts for a given identified peptide were recorded. Carbamidomethyl (+57 on C) was considered a fixed modification, while deamidation (+1 on N/Q), oxidation (+16 on M), phosphorylation (+80 on S/T/Y) and acetylation (+42 on K/N-terminal M) were considered as variable modifications.

2.5. Electrophoretic mobility shift assay (EMSA)

Recombinant ELK-1-His proteins (25/50 ng) were incubated with Cy5-labelled E74 probe for 10 min at room temperature alongside 1 μg poly(dI-dC) and 5.4 μg herring sperm DNA as non-specific competitors. Complexes were resolved on native 5% polyacrylamide gels and visualised on an Amersham Typhoon Biomolecular imager. When *in vitro* phosphorylation prior to EMSA was required, ELK-1 protein was incubated with active ERK2 [27] in PP buffer (25 mM Tris-HCl pH 7.2, 10 mM $MgCl_2$, 1 mM DTT, 250 μM ATP) for 1 h at 37°c.

2.6. Protein interaction assay

GST-AR-N-His (1 μg) on glutathione agarose beads was pre-blocked with 0.1% w/v BSA (in HBS) for 1 h, washed with HBS and incubated with ELK-1 protein (1 μg) for 2 h at room temperature. Beads were washed and resolved by SDS-PAGE gradient gel (5–20%), transferred to nitrocellulose membrane and submitted to immunoblot.

2.7. Co-immunoprecipitation (Co-IP) assay

HEK293T cells were transfected with pCMV5-ELK-1-His and pCMV5-AR-FLAG in 6-well dishes using TransIT-LT1 transfection reagent as per manufacturer's instructions (Mirus Bio). The following day cells were serum-starved and stimulated, before lysis in Co-IP buffer (40 mM HEPES pH 7.4, 150 mM NaCl, 1% v/v NP40), sonication for 10 s (amplitude 25 Hz) followed by centrifugation (14,000 rpm, 4 °C, 15 min). Supernatants were incubated with anti-FLAG M2 affinity gel (Sigma-Aldrich) for 2 h at room temperature, beads were washed, eluted proteins were resolved by SDS-PAGE gradient gel (5–20%), transferred to nitrocellulose membrane and submitted to immunoblot.

3. Results

3.1. ELK-1 phosphorylation and acetylation modifications vary depending on protein origin

To produce comparable protein preparations, cDNA encoding C-terminally His-tagged full-length human ELK-1 was cloned into vectors appropriate for each expression system of interest (Fig. 1b). Proteins were generated through transient transfection in human cells (HEK293T), viral infection in insect cells (Sf9) and transformation and induction in bacterial cells (*E. coli* SG13009). Following lysis of cells, ELK-1-His was purified through immobilised metal affinity chromatography (IMAC). From denaturing SDS-PAGE analysis of purified proteins, we noted clear differences between protein samples analysed in parallel, with an apparent faster migration of bacterially produced ELK-1 suggestive of lower denatured molecular weight (Fig. 1c). Also of note was the apparent homogeneity in bacterial and insect cell-derived proteins, indicated by a single sharp gel band, compared to a more dispersed band for the human cell-derived ELK-1 protein, potentially indicating diversity in observed molecular weight due to PTM heterogeneity.

To determine whether this was the case, equal amounts of the purified proteins were submitted to immunoblot against a panel of antibodies that recognise PTMs. ELK-1 phosphorylation is well-characterised in the C-terminal TAD, with S383 and S389 established as positive indicators of transcriptional activation following mitogen stimulation [8–10]. Commercial antibodies are available for phosphoS383-ELK-1, and as expected gave a strong signal against human cell-derived purified ELK-1, consistent with endogenous ERK expression and other mitogen activated protein kinases (MAPKs) in HEK293T cells (Fig. 2a). A weaker signal was seen in insect cell-derived ELK-1 protein, with no signal detected with the bacterially-derived protein, rationally reflecting the lack of appropriate PTM machinery in *E. coli* [5]. Tyrosine phosphorylation in ELK-1 is not well studied, although it has been documented in large proteomic datasets [28]. Immunoblot for phospho-Y also gave (weak) reactivity with human cell-derived ELK-1, with no detected reactivity in the other samples. Lysine acetylation is undocumented in ELK-1, although ELK-1 is known to associate with p300/CBP [29–31]. Interestingly, immunoblot against acetyl-K gave the strongest signal with the Sf9-derived purified ELK-1 protein, with weaker reactivity for the HEK293T-derived ELK-1 and very faint detection of *E. coli*-derived protein.

To complement immunoblot data, we used MS/MS to identify PTM sites in purified ELK-1 proteins. As expected, no phosphorylation sites were found in the bacterial preparation of ELK-1, while multiple sites were identified in both insect and human-derived protein at sub-stoichiometric levels (Fig. 2b/Table S1). Of these, S324 has been previously characterised as an ERK target and is associated with transcriptionally active ELK-1, while S304 has been documented in a proteomic study of mitotic phosphorylation events [10,32]. Of note, S383 (detected by immunoblot) and S389 were absent from MS/MS datasets due to a lack of sequence coverage of the ELK-1 C-terminus.

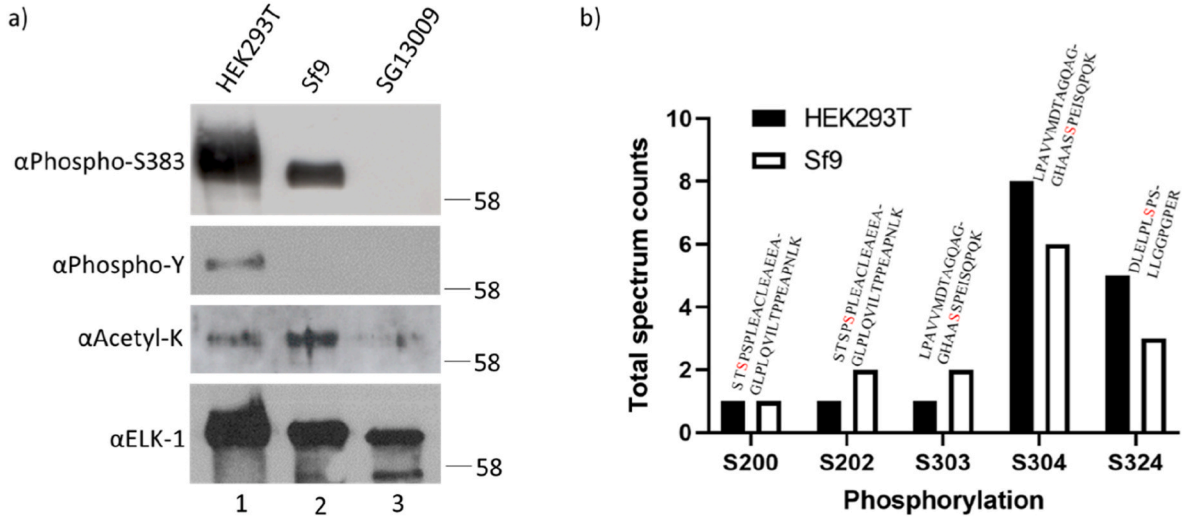

Fig. 2. Comparison of recombinant ELK-1 PTM profiles. **a)** Immunoblot for a panel of PTM markers as indicated on equal protein loads of ELK-1 preparations (MW markers indicated right of blot image, kDa). **b)** HEK293T and Sf9-derived ELK-1 phosphorylation sites identified by MS/MS, with bars representing total spectrum counts for each peptide. Tryptic peptide is labelled above graph, with modification site highlighted red.

Regarding lysine acetylation, only low-level peptide modifications could be confirmed by mass spectrometry with no overlap of sites between expression systems (data not shown).

3.2. Differential PTM profiles associated with variable DNA and AR binding efficacies in ELK-1

Having established differential candidate PTM profiles for the various purified ELK-1 proteins, we assessed their performance in

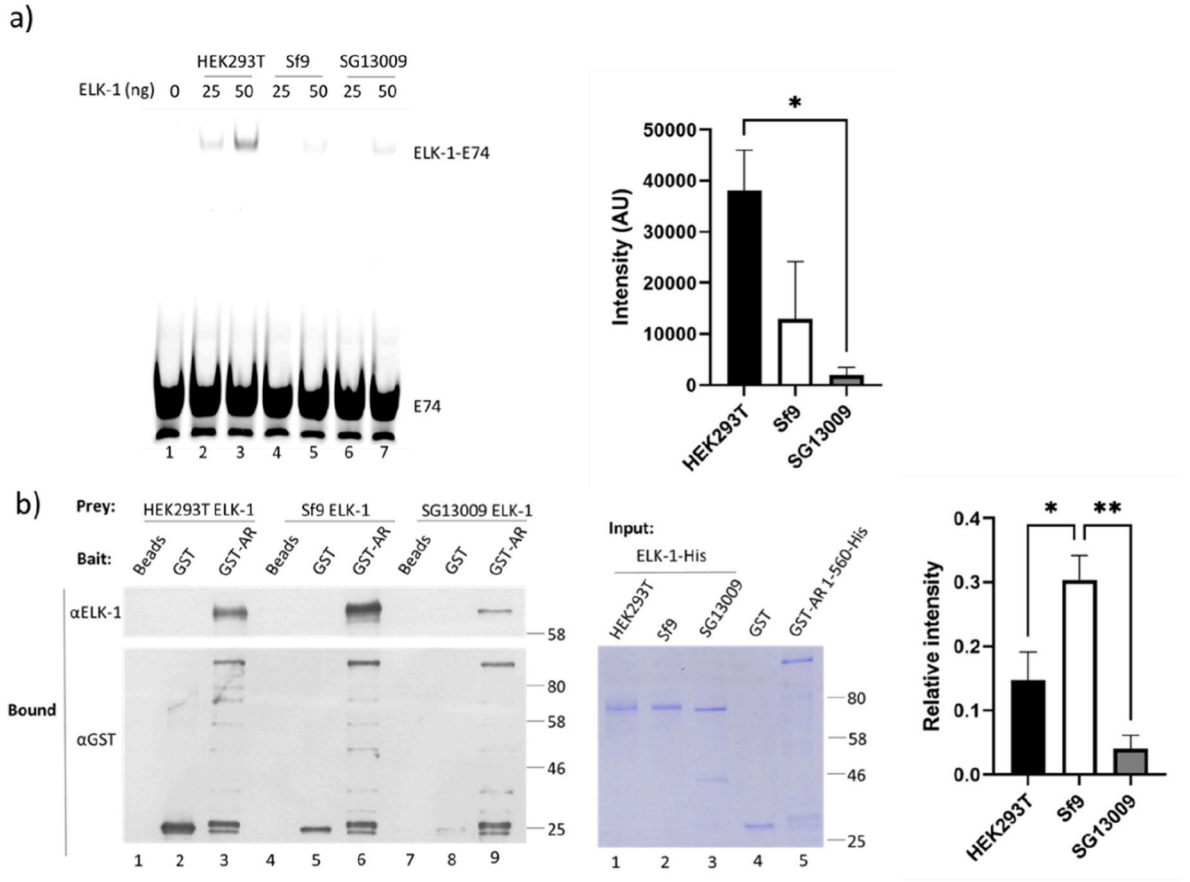

Fig. 3. Comparison of recombinant ELK-1 proteins in functional assays. **a)** ELK-1 binding to fluorescent Cy5-E74 DNA probe. Graph denotes densitometry measurements of bound ELK-1 (50 ng) displayed as averages of three independent experiments (± SEM). **b)** ELK-1 binding to immobilised GST-tagged AR-N (1–560; MW markers indicated right of blot/gel image, kDa). Graph denotes densitometry measurements of bound ELK-1 normalised to total ELK-1 load displayed as averages of three independent experiments (± SEM). Statistical analyses were performed with one-way ANOVA; significance is reported in figures by $*P < 0.05$, $**P < 0.01$.

functional (*in vitro* binding) assays. Due to its role as a transcription factor, ELK-1 DNA-binding was first explored. A particularly high affinity DNA-binding site for the ETS domain is E74 (5′-ACCGGAAGT-3′), first characterised as a target for the ETS protein E74 in *Drosophila* [33]. Human cell-derived ELK-1 bound E74 probe by EMSA significantly more readily than *E. coli*-derived protein, which only showed very weak interactions (Fig. 3a). This likely reflects the contrasting C-terminal TAD phosphorylation levels (e.g. S383) in these ELK-1 preparations, which is known to enhance DNA-binding, although it could also be influenced by other factors unique to each expression system. Attenuated DNA-binding of ELK-1 produced in *E. coli* (and Sf9 cells) in the absence of subsequent *in vitro* phosphorylation is in line with previous reports [26,34]. To confirm functionality, bacterially-prepared ELK-1 was phosphorylated *in vitro* with recombinant, active ERK, whereafter it bound the E74 DNA probe more efficiently (Fig. S1).

Protein-protein interactions of ELK-1 are also key to its function and are of increasing relevance to carcinogenesis. ELK-1 acts as a tethering protein for AR in PC, recruiting it to target genes and promoting cell growth [21]. The intrinsically disordered N-terminal of AR (AR-N) is sufficient for this interaction, which is mediated through the MAPK-docking motifs in ELK-1 (D/F - Fig. 1a) [2,21,35],. We found that binding to GST-AR-N in pulldown assays was most pronounced with Sf9-expressed ELK-1, with lower levels of binding with HEK293T-derived ELK-1 and much reduced binding associated with the *E. coli*-derived protein (Fig. 3b). Unlike DNA-binding, the ELK-1/AR interaction is reported to be independent of TAD phosphorylation [2,21]. Our data is consistent with this notion, with no apparent correlation between phosphorylation status of ELK-1 and AR-N interaction.

3.3. Mitogen stimulation of HEK293T cells promotes lysine acetylation of ELK-1 at known sites of other PTMs

Recognising that acetylation of ELK-1 protein may be a hitherto unexplored regulatory PTM, we manipulated HEK293T cells transfected with ELK-1-His through serum starvation, followed by mitogen stimulation. Here, purified ELK-1 from stimulated cells showed increased phosphorylation (most prominently at S304), along with substantial acetylation at K254 and K271, detected by MS/MS, when compared with protein from serum-starved cells (Fig. 4a and b). Interestingly, K254 is a *bona fide* ELK-1 SUMOylation site [36]. Moreover, there was proteomic evidence of low-level acetylation at K35, which we have previously shown to be a regulatory ubiquitination site [19]. Evaluation of ubiquitination sites of ELK-1 identified a single peptide containing ubiquitinated K59 and a single modified K35 peptide from serum-starved and mitogen-stimulated samples respectively. These data point to potential crosstalk and/or antagonism within the ELK-1 PTM landscape that has been overlooked to date (Fig. 4c). The increase in acetylation seen in mitogen-stimulated cells indicates the potential for further PTM-driven regulation of ELK-1 function. Initial experiments suggest that acetylation does not impact on ELK-1-AR interactions (Fig. S2), consistent with their independence from the mitogen signalling axis [2,21].

4. Discussion

The data presented illustrate variations in protein functional activity depending on the expression system used, alongside differences in PTM landscape, with ELK-1 as our paradigm (Table 1). With regard to PTMs,

Table 1
Summary of differences between human, insect and bacterial cell-derived preparations of ELK-1. PTM assessment based on immunoblot analysis.

		Human	Insect	Bacterial
PTM	Phosphorylation (S383)	+++	++	
	Phosphorylation (Y)	++		
	Acetylation (K)	++	+++	+
Function	DNA binding (E74)	+++	++	+
	Protein binding (AR)	++	+++	+

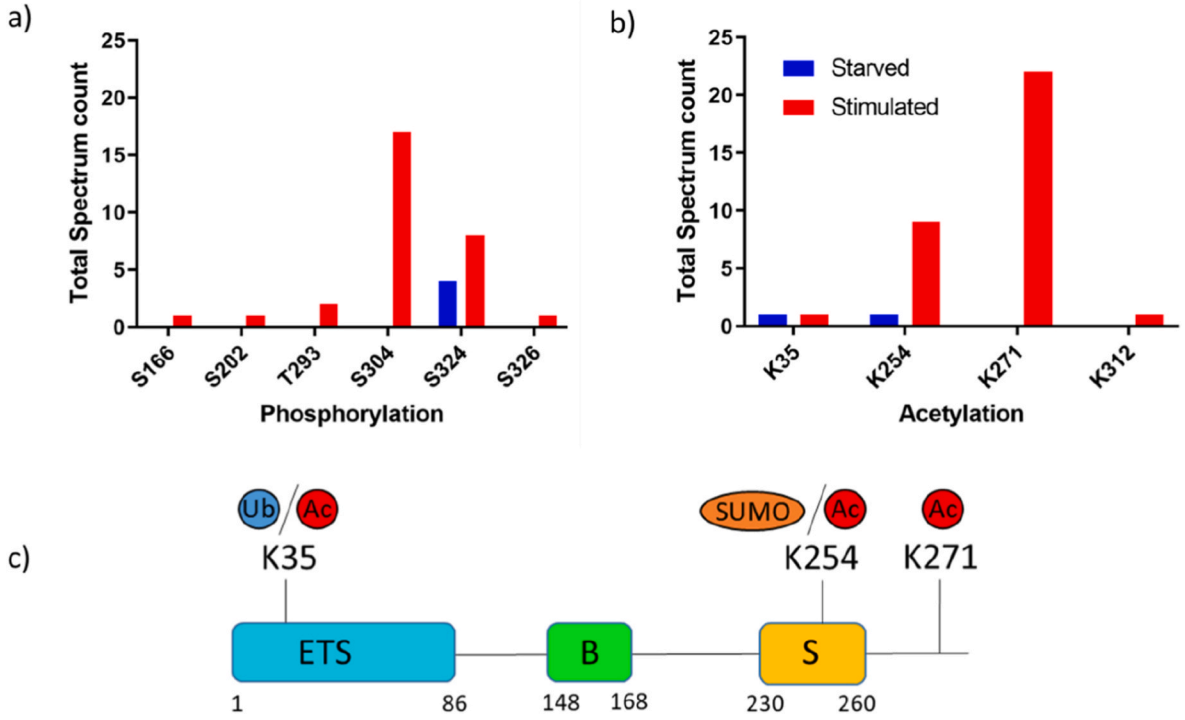

Fig. 4. Impact of mitogen stimulation on ELK-1 PTM profile. **a)** Phosphorylation sites identified by MS/MS in transfected human ELK-1 from serum-starved (blue) and serum/TPA stimulated (red) HEK293T cells, with bars representing Total Spectrum Counts (Scaffold) for each indicated tryptic peptide carrying the indicated modified residue. **b)** As for a) for acetylation sites. **c)** Schematic showing acetylation sites identified in purified ELK-1 by MS/MS and overlap with previously identified ubiquitination and SUMOylation sites.

phosphorylation of ELK-1 prepared from HEK293T cells was entirely rational given the mammalian background. Higher phosphorylation levels correlated with increased DNA interactions for protein isolated from mammalian cells (Figs. 2 and 3a). Replicating TAD phosphorylation on ELK-1 isolated from *E. coli* requires post-purification *in vitro* phosphorylation (Fig. S1) or possibly use of phospho-mimetic mutants [5,27,34,37]. This would not replicate some poorly studied ELK-1 phosphorylation sites however, which may also influence protein function, such as those mediated by tyrosine kinases [28].

Lysine acetylation of transcription factors can have various functional impacts, including promoting or abrogating DNA binding and regulating protein turnover [38]. We present evidence of lysine acetylation of ELK-1, a hitherto unreported modification. ELK-1 acetylation levels appeared highest in protein purified from Sf9 cells, with the reasons behind this unclear (Fig. 2a). A previous study found that global lysine acetylation in Sf9 cells was significantly lower than in mammalian AA8 cells (a CHO derivative) [39]. However, this does not account for how these cells react to the stress of viral infection (used here for ELK-1-His expression) and subsequent overexpression of a foreign transcription factor. Dynamic acetylation events in human lung fibroblasts were found to be important for defence responses and viral replication in response to human cytomegalovirus infection [40]. Plant pathogens are also known to encode acetyltransferase enzymes that can modify host proteins and modulate immune responses [41]. Hence it seems plausible that baculoviral infection of insect cells could inadvertently prompt acetylation events on latent, ectopically expressed protein, of potential relevance to expression of other proteins in this system.

Whether the relatively high level of ELK-1 acetylation seen in Sf9 cell-derived protein mimics any physiological modification of the protein remains to be clarified. Notably, ELK-1 from serum-stimulated human cells showed increased acetylation by immunoblot and MS/MS compared with starved cells, including at consensus SUMOylation and ubiquitination sites (Fig. 4/S2). The possibility of competition with repressive ubiquitin/SUMO modifications suggests that acetylation could be a positive marker for ELK-1 activity following mitogen induction in the regulation of cell proliferation. Increased lysine acetylation may augment ELK-1 de-ubiquitination and/or de-SUMOylation, which correlate with an increase in TAD phosphorylation. Competition between acetylation and other PTMs is well documented, particularly with regard to the tumour suppressor p53, where p300-mediated acetylation in the C-terminal regulatory region can directly block ubiquitination to protect against proteasomal degradation, and SUMOylation can prevent acetylation at adjacent lysine residues to inhibit DNA binding [42,43]. Further study is required to elucidate whether similar PTM interplay exists in the ELK-1 nexus.

ELK-1 interaction with AR-N protein showed variability depending on the origin of the ELK-1 protein, with bacterially-expressed ELK-1 found to be a weak binder of AR-N compared to the other samples (Fig. 3b). Mitogen-stimulated lysine acetylation and serine/threonine phosphorylation of ELK-1 appeared not be directly involved in regulating this interaction (Fig. S2), although involvement of other PTMs cannot be ruled out. Issues with correct protein folding as a result of lack of PTM seem unlikely, due to the inherent disorder in ELK-1 regions important for AR docking, and that structured domains of ELK-1 readily fold and function correctly when expressed in isolation in *E. coli* [12,34]. A complicating factor of this could be the provenance of the AR-N protein itself, which was produced in *E. coli* (Rosetta) for this study, so it is feasible that ELK-1-AR binding could be subject to further regulation through PTMs on AR. Indeed, an area for future investigation is AR acetylation, recently described as an important step for its activation [44].

Our data highlight the danger of oversimplification when viewing recombinant expression systems as a route to soluble protein production for functional studies. The use of different expression backgrounds, all of which were successful in producing full-length purified proteins, nonetheless resulted in clear variations in binding efficacy of ELK-1 to DNA and a known disease-relevant protein cofactor, AR. More generally, this reinforces the importance of the source of purified proteins in the biochemical analysis of protein-protein interactions. Unravelling the complex PTM network governing the function of a protein of interest is pivotal to understanding its roles in human health and disease. With this information, careful considerations can be made over the best expression system to generate recombinant protein with optimal biochemical activity, targeted to assay requirements.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

Abbreviations

PTM	Post-translational modification
ETS	E-twenty-six/E26
TCF	Ternary complex factor
ERK	Extracellular signal-regulated kinase
TAD	Transactivation domain
SRF	Serum Response Factor
SRE	Serum Response Elements
PC	Prostate cancer
AR	Androgen receptor
TPA	12-*O*-tetradecanoylphorbol-13-acetate
HBS	HEPES-buffered saline
GST	Glutathione S-transferase
EMSA	Electrophoretic mobility shift assay
Co-IP	Co-immunoprecipitation
IMAC	Immobilised metal affinity chromatography
MAPK	Mitogen activated protein kinase
MS/MS	Tandem mass spectrometry

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.pep.2022.106216.

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