ELM 2016—data update and new functionality of the eukaryotic linear motif resource

Holger Dinkel¹, Kim Van Roey², Sushama Michael¹, Manjeet Kumar¹, Bora Uyar¹, Brigitte Altenberg¹, Vladislava Milchevskaia¹, Melanie Schneider³, Helen Kühn³, Annika Behrendt³, Sophie Luise Dahl³, Victoria Damerell³, Sandra Diebel³, Sara Kalman³, Steffen Klein³, Arne C. Knudsen³, Christina Mäder³, Sabina Merrill³, Angelina Staudt³, Vera Thiel³, Lukas Welt³, Norman E. Davey⁴, Francesca Diella¹ and Toby J. Gibson¹,*

¹Structural and Computational Biology, European Molecular Biology Laboratory, Meyerhofstrasse 1, 69117 Heidelberg, Germany, ²Health Services Research Unit, Operational Direction Public Health and Surveillance, Scientific Institute of Public Health (WIV-ISP), 1050 Brussels, Belgium, ³Ruprecht-Karls-Universität, Heidelberg, Germany and ⁴Conway Institute of Biomolecular and Biomedical Sciences, University College Dublin, Dublin 4, Ireland

Received October 11, 2015; Revised November 4, 2015; Accepted November 5, 2015

ABSTRACT

The Eukaryotic Linear Motif (ELM) resource (http://elm.eu.org) is a manually curated database of short linear motifs (SLiMs). In this update, we present the latest additions to this resource, along with more improvements to the web interface. ELM 2016 contains more than 240 different motif classes with over 2700 experimentally validated instances, manually curated from more than 2400 scientific publications. In addition, more data have been made available as individually searchable pages and are downloadable in various formats.

INTRODUCTION

Short Linear motifs (SLiMs) are small protein-interaction-mediating modules that have unique properties (1,2). They play an important role in biological systems (3–6) and the analysis of motifs in protein sequences remains an important step in protein research. The ELM database provides manually curated classes of motifs as well as instances thereof. Furthermore, it provides a web interface that allows users to explore possible instances of annotated classes in proteins of interest. More than 10 years since its inception (7,8) it remains a popular resource being continually used by scientists worldwide. It has proved invaluable for investigating interactions mediated by short linear motifs, be it for individual proteins (9–11), large-scale analyses (12–14), algorithm development (15), prediction of novel motif-mediated interactions (16) or investigation of host–virus interactions (17–20). Here we give an overview of the latest developments and new features introduced to the ELM resource (see Figure 1) since the last update (21).

RESOURCE DESCRIPTION

The ELM resource provides two main services, a database of short linear motif annotation and a tool that uses this information to explore possible instances of motifs in any given protein sequence. The main database content are annotations of motif classes, hand-curated from the scientific...
Figure 2. Screenshot of the ELM home page showing autocompletion of keyword search and expanded menu bar. The dropdown menu lists individual pages of ELM database content; the top right shows a dropdown of the autocompletion search for the keyword ‘pp2’, different content type is highlighted in different colors (ELM classes: orange, ELM instances: green, ELM class candidates: blue); a click on a link directs to the details page of the entry.

Figure 3. Percentages of ELM classes (outer ring) and instances (inner ring) by type.

An ELM motif class is described by a unique regular expression pattern, such as ‘...([ST])P.’ (meaning any three residues followed by a serine or threonine, followed by proline and another wildcard residue) for the DOC_WW_Pin1_4 class. Ideally, each motif class has multiple example instances of this motif annotated, whereby an instance is described as a match to the regular expression pattern of the ELM motif class in a protein sequence. For each instance entry, ideally, multiple sources of experimental evidence are recorded (identifying participant, detecting motif presence and detecting interaction), and, following annotation best practices, a reliability score is given by the annotator.

NEW FEATURES AND FUNCTIONALITY

Interface

The HTML interface has been updated and new functionality has been added. A multi-tier navigation menu has been implemented to provide improved accessibility to the individual pages. Also, on each page, a unified search box is available, providing auto-completion and faster access to the ELM database content via simple keyword search (see Figure 2).

Several new webpages have been introduced to allow easier access to the database content. There are now individual pages for interaction domains, motif-mediated switches, PDB structures, ELM methods and GO terms. This allows easy access to individual content searching and selecting data by user provided keywords, fostering data dissemination and re-use.

Database content

The ELM database has been updated and existing data types have been enriched with more data, see Tables 1 and 2, Figures 1 and 3 for an overview of data on ELM classes and instances, as well as Figures 4 and 5 for information about the taxonomic distribution of ELM instances and the

literature and enriched with links to resources such as PSI-MI (22), UniProt (23), GO (24), PDB (25), switches.ELM (6), Interpro (26), iELM (27), Pfam (28) and KEGG (29).
Table 1. Summary of data stored in the ELM database

| Functional sites | ELM classes | ELM instances | PDB structures | GO terms | PubMed links |
|------------------|-------------|---------------|----------------|----------|--------------|
| Total            | 159         | 246           | 2702           | 348      | 549          |
| By category      |             |               |                |          |              |
| LIG              | 137         | Human         | 1594           | Biological Process | 283 From class | 1174 |
| MOD              | 31          | Mouse         | 253            | Cellular Compartment | 119 From instance | 1746 |
| DEG              | 25          | Rat           | 130            |          |              |
| DOC              | 22          | Yeast         | 94             |          |              |
| TRG              | 20          | Fly           | 90             |          |              |
| CLV              | 11          | Other         | 541            | Molecular Function | 147          |

1 as of November 2015.

Database and web server optimization

The ELM resource is implemented using a PostgreSQL relational database as a backend to store all annotations and associated data, while the front-end web interface makes use of the Django web-framework. Recent improvements include an updated annotation system allowing easy annotation and correction of entries. This aids annotators in inserting novel entries and updating existing ones. Figure 1 illustrates how this annotation system has helped increasing the database content: since its implementation (in the year 2014) many new ELM classes and instances have been annotated, and a large number of classes and instances have been revised.

The high degree of data integration and interconnectedness caused some ELM database queries to become slow. To remedy this and thus increase user experience, an HTTP Cache/Reverse Proxy has been employed caching rendered HTML pages, which significantly speeds up page delivery and increases user experience.

Downloads

The data annotated in the ELM database is freely available to the scientific community and the ELM team tries to make this data as easily accessible as possible. Several new pages are now available providing more download formats/options; the best starting point for looking for ELM downloads is the web page http://elm.eu.org/downloads.html. Novel pages include the experimental methods used during annotation, the PDB structures associated with motif–domain interactions, or all linked GO-terms. All of these are also available for download in computer-parseable tab-separated format. Also, a simple timestamp has been implemented, which allows clients to update their data only when newer data is available. Further formats and options can be implemented upon request; the authors welcome suggestions to implement user-suggested features.

SHORT LINEAR MOTIFS IN BIOLOGICAL PATHWAYS

Relevance of linear motifs is well known in signaling pathways. Furthermore, accumulation of more experimental evidence demands a systematic analysis of all biological pathways for the presence of linear motifs. The ELM resource assists in this endeavor by providing a distinct color mapping of different ELM classes on the proteins in-
Table 2. 49 novel1 ELM classes that have been added since the last ELM publication (21), together with the number of associated instances (middle column) and a short description

| ELM class identifier       | Instances | ELM class description                                                                                                                                                                                                 |
|----------------------------|-----------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| DEG_Kelch,KLHL3_1          | 4         | An acidic degron motif present in wnk kinases necessary to interact with kelch domain of KLHL2 and KLHL3 proteins for efficient ubiquitination degradation.                                                                |
| DEG_Kelch,Keap1_1          | 13        | Motif that binds to the kelch domain of KEAP1 with high affinity. This high affinity motif is required for the efficient recruitment of target proteins to the Cul3-based E3 ligase.                        |
| DEG_Kelch,actinfilin_1     | 1         | A hydrophobic degron motif present in some kainate receptors necessary to interact with kelch domain of actinfilin protein for efficient ubiquitination and degradation.                                       |
| DEG_Nend,UBRbox_1          | 0         | N-terminal motif that initiates protein degradation by binding to the N-box of N-recognins. This N-degron variant comprises a bulky hydrophobic residue as destabilizing residue.                        |
| DEG_Nend,UBRbox_2          | 0         | N-degron variants comprise different N-terminal residues. Type I destabilizing residues can either occur as primary or secondary and tertiary destabilizing amino acids, which can be conjugated to a primary destabilizing residue. |
| DEG_Nend,UBRbox_3          | 0         | Destabilizing residues, which are positively charged amino acids directly recognized by N-recognins, or as a regulatory CNB subunits.                                                                                       |
| DEG_Nend,UBRbox_4          | 8         | N-degron motif present in extracellular of some Wnt antagonists recognized by the N-terminal domain of DVL in Wnt pathway.                                                                                              |
| DEG_Nend_UBRbox_2          | 0         | N-terminal motifs that initiate protein degradation by binding to the UBR-box of Mtr4.                                                                                                                                |
| DEG_Nend_UBRbox_3          | 0         | N-recognins. Four different N-degron variants comprise different N-terminal residues. Type 1 destabilizing residues can either occur as primary or secondary and tertiary destabilizing amino acids, which can be conjugated to a primary destabilizing residue. |
| DEG_Nend_UBRbox_4          | 8         | N-terminal motifs that initiate protein degradation by binding to the UBR-box of Mtr4.                                                                                                                                |
| DEG_SPOP_SBC_1             | 8         | The S/T rich motif known as the SPOP-binding consensus (SBC) of the MATH-BTB protein, SPOP, is present in substrates that undergo SPOP/Cul3-dependant ubiquitination.                                               |
| DOC_CKS1_1                 | 8         | Phospho-dependent motif that mediates docking of CDK substrates and regulators to cyclin-CDK-bound Cks1.                                                                                                               |
| DOC_GSK3_Axin_1            | 6         | Docking motif present in Axin protein binds the GSK-3β kinase and aids the phosphorylation of components in the APC destruction complex.                                                                             |
| DOC_PPI1_MyPhoNE_1         | 9         | Docking motif that binds to the catalytic subunit of Protein Phosphatase 1 (PP1c).                                                                                                                                 |
| DOC_PPI1_SILK_1            | 14        | Protein phosphatase 1 catalytic subunit (PP1c) interacting motif that often cooperates with and is located N-terminal to the RXVX motif to dock proteins to PP1c.                                                      |
| DOC_PPP2A_KARD_1           | 1         | Protein Phosphatase 2A (PP2A)-binding motif found in BubR1 for docking to the regulatory subunit B56 of PP2A.                                                                                                        |
| DOC_PPP2B_LxvP_1           | 8         | Docking motif in calcineurin substrates that binds at the interface of the catalytic CNA and regulatory CNB subunits.                                                                                                 |
| DOC_USP7_UBL2_3            | 0         | The USP7 CTD domain binding motif variant based on the ICP0 and DNMT1 interactions.                                                                                                                                  |
| LIG_Axin,LRP6_1            | 0         | Motif in LRP6, which in its phosphorylated form binds Axin in a pseudo-substrate manner.                                                                                                                           |
| LIG_CID_NIM_1              | 1         | The NIM motif in Trf4 interacts with the CTD-interacting domain (CID) of Nrd1.                                                                                                                                        |
| LIG_CNOT1_NIM_1            | 10        | The CNOT1-interacting motif (NIM) found in Nanos proteins mediates recruitment of the CCR4-NOT deadenylase complex.                                                                                                   |
| LIG_CaMK_CASK_1            | 6         | Motif mediating binding to the calmodulin-dependent protein kinase (CaMK) domain of the membrane protein CASK/Lim2.                                                                                                   |
| LIG_DCNL_PONY_1            | 2         | DCNL PONY domain binding motif variant based on the UBE2M and UBE2F interactions.                                                                                                                                   |
| LIG_EF_ALG2_2ABM_1         | 9         | This isofrom-specific ALG-2-binding motif binds to the EF hand domains of the proapoptotic Ca2⁺-binding protein.                                                                                                   |
| LIG_EF_ALG2_2ABM_2         | 3         | ALG-2 protein in a calcium-dependent manner.                                                                                                                                                                       |
| LIG_FZD_DVL1_DPD_1         | 0         | A short internal motif near the C-terminus of Frizzleds, which interacts with the PDZ domain of DVL in Wnt pathway.                                                                                               |
| LIG_GBD_WASP_1             | 4         | A hydrophobic motif of double function – it acts as an autoinhibitory element of the GTPase-binding domain (GDB), as well as mediating the protein’s interactions with the Arp2/3 complex.                        |
| LIG_GSK3_LRP6_1            | 8         | Motif present five times on membrane receptor LRP6, responsible for GSK3 binding and inhibition when phosphorylated.                                                                                             |
| LIG_LIR_Apic_2             | 1         | Apicomplexa-specific variant of the canonical LIR motif that binds to Atg8 protein family members.                                                                                                                 |
| LIG_LIR_2Gen_1             | 21        | Canonical LIR motif that binds to Atg8 protein family members to mediate processes involved in autophagy.                                                                                                           |
| LIG_LIR_LC3C_4             | 1         | Non-canonical variant of the LIR motif that binds to Atg8 protein family members to mediate processes involved in autophagy.                                                                                      |
| LIG_LIR_Nem_3              | 1         | Nematode-specific variant of the canonical LIR motif that binds to Atg8 protein family members.                                                                                                                        |
| LIG_LRP6_Inhibitor_1       | 0         | Short motif present in extracellular of some Wnt antagonists recognized by the N-terminal β-propeller domain of LRPS/6 and thus inhibits the Wnt pathway.                                                            |
| LIG_Mtr4_Air2_1            | 3         | This motif on Air2 interacts with the DEExH core of Mtr4, forming a part of the nucleus-located TRAMP complex.                                                                                                |
| LIG_Mtr4_Tr4f_1            | 4         | This motif on Trf4 interacts with the DEExH core of Mtr4, forming a part of the nucleus-located TRAMP complex.                                                                                                |
| LIG_Mtr4_Tr4f_2            | 3         | This motif on PAPD5 interacts with the DEExH core of SKIV2L2, forming a part of the nucleus-located TRAMP complex.                                                                                                |
| LIG_Pex14_3                | 1         | Motif in Pex5 binding the N-terminal domain (NTD) of Pex14                                                                                                                                                    |
| LIG_Pex14_4                | 0         | Fungal motif in Pex5 interacting with the N-terminal domain of Pex14                                                                                                                                             |
| LIG_RPAC_Fungi             | 1         | Fungi version of the RPA interacting motif.                                                                                                                                                                       |
Table 2. Continued

| ELM class identifier | Instances | ELM class description |
|----------------------|-----------|-----------------------|
| LIG\_RPA\_C\_Insects | 0         | Insect version of the RPA interacting motif. |
| LIG\_RPA\_C\_Plants  | 0         | Plant version of the RPA interacting motif, which is located on DNA replication and repair proteins UNG2, XPA, TIPIN, SMARCAL1 and RAD14 and interacts with Replication Protein A (RPA), a DNA binding protein. |
| LIG\_RPA\_C\_Vert    | 4         | The RPA interacting motif is located on DNA replication and repair proteins UNG2, XPA, TIPIN, SMARCAL1 and RAD14 and interacts with Replication Protein A (RPA), a DNA binding protein. |
| LIG\_SUFU\_1         | 5         | A hydrophobic motif in GLI transcription factors required for binding to SUFU protein, which inhibits their activity and hence negatively regulates hedgehog signalling. |
| LIG\_UBA3\_1         | 2         | UBA3 adenylation domain binding motif variant based on the UBE2M and UBE2F interactions. |
| LIG\_WD40\_WDR5\_VDV\_1 | 3     | This WDR5-binding motif binds to a cleft between blades 5 and 6 of the WD40 repeat domain of WDR5, opposite |
| LIG\_WD40\_WDR5\_VDV\_2 | 2     | of the Win motif-binding site, to mediate assembly of histone modification complexes. |
| LIG\_WD40\_WDR5\_WIN\_1 | 7     | Known as the Win (WDR5 interaction) motif, this peptide contains an invariant arginine residue that inserts into the central tunnel of the WD40 repeat domain of WDR5 to mediate assembly of histone modification complexes. |
| LIG\_WD40\_WDR5\_WIN\_2 | 4     | Surrounding this arginine are small residues that fit tightly at the entrance of the arginine-binding pocket. |
| LIG\_WD40\_WDR5\_WIN\_3 | 3     | Inverted version of SUMOylation motif recognized for modification by SUMO-1 |

\(^1\) as of November 2015.

Figure 6. ‘Wnt Signaling pathway’ from KEGG (29) (ID hsa04310). The color scheme has been modified for the cases having more than one ELM class in order to provide additional clarity. The original figure can be reproduced on the ELM web server (http://elm.eu.org/pathways/index.html?q=wnt)

Involves in different pathways, information of which is obtained from KEGG (29). We have used the Wnt signaling pathway (hsa04310) to illustrate this feature (Figure 6).

Signaling in this pathway starts from Wnt proteins that transduce the signal from the extracellular part of the cell to the interior via the Frizzled receptor, which in turn transmits it to other regulatory proteins. A key component of Wnt signaling is β-catenin which needs to accumulate in the cytoplasm in order to be translocated into the nucleus, where it subsequently induces a cellular response via gene transduction (33). This accumulation however is regulated by APC/Axin1 destruction complex in which β-catenin is
ubiquitylated (34). Thereafter, modified β-catenin is degraded via proteasomal machinery. Short linear motifs play a prominent role in this process as exemplified by the presence of at least four different motifs in β-catenin (annotated at the ELM resource): It contains modification sites for Ca-
sein kinase 1 (CK1) and glycogen synthase kinase 3 (GSK3) and the sequential modification of these sites by CK1 and GSK3 generates a phosphodegron at the N-terminus of this protein. This phospho-regulated degron acts as a recognition site for β-TrCP, a subunit of the SCF-β-TrCP E3 ligase enzyme (35). After binding to the activated degron, β-TrCP ubiquitylates β-catenin, which gets subsequently degraded by the proteasomal machinery (36). Among other classes, β-catenin contains ligand binding (LIG) motifs for 14-3-3 proteins and the binding of 14-3-3 along with Chibby protein has been suggested to facilitate the nuclear export of β-catenin, which puts an end to its signaling (37). The presence of each of these motifs is necessary for modulation of this pathway and the regulation of β-catenin by different motif classes clearly underlines the important role of short linear motifs in signaling pathways.

CONCLUSION AND FUTURE DIRECTIONS

The number of motifs that have been experimentally validated up to today is still small, with annotated instance numbers in the low thousands, compared to the estimated total number, which might exceed a million (38); hence, we expect many more motifs and motif-mediated interactions to be discovered (for guidelines on motif discovery see (39)). The ELM resource will continue to provide support to the scientific community with a repository of high quality annotations and facilitate the linear motif analysis of protein sequences.

ACKNOWLEDGEMENT

The authors would like to thank the users of the ELM resource for their longstanding support as well as colleagues and annotators for their contributions and feedback to the ELM database.

FUNDING

European Community's Seventh Framework Programme FP7/2009 (SyBoSS) [242129 to K.V.R.]; EMBL International PhD Program (to B.U.); SFI Starting investigator Research Grant [13/SIRG/2193 to N.E.D]. Funding for open access charge: EMBL.

Conflict of interest statement. None declared.

REFERENCES

1. Davey, N. E., Van Roey, K., Weatherritt, R. J., Toedt, G., Uyar, B., Altenberg, B., Budd, A., Diella, F., Dinkel, H. and Gibson, T. J. (2012) Attributes of short linear motifs. Mol. Biolyst., 8, 268–281.
2. Van Roey, K., Uyar, B., Weatherritt, R. J., Dinkel, H., Seiler, M., Budd, A., Gibson, T. J. and Davey, N. E. (2014) Short linear motifs: ubiquitous and functionally diverse protein interaction modules directing cell regulation. Chem. Rev., 114, 6733–6778.
3. Dyson, H. J. and Wright, P. E. (2005) Intrinsically unstructured proteins and their functions. Nat. Rev. Mol. Cell Biol., 6, 197–208.
The Protein Data Bank: a computer-based archival file for macromolecular structures. *J. Mol. Biol.*, **112**, 535–542.

26. Mitchell, A., Chang, H.-Y., Daugherty, L., Fraser, M., Hunter, S., Lopez, R., McAnulla, C., McMenamin, C., Nuka, G., Pesseat, S. *et al.* (2015) The InterPro protein families database: the classification resource after 15 years. *Nucleic Acids Res.*, **43**, D213–D221.

27. Weatheritt, R.J., Jehl, P., Dinkel, H. and Gibson, T.J. (2012) iELM—a web server to explore short linear motif-mediated interactions. *Nucleic Acids Res.*, **40**, W364–W369.

28. Punta, M., Coggill, P.C., Eberhardt, R.Y., Mistry, J., Tate, J., Boursnell, C., Pang, N., Forslund, K., Ceric, G., Clements, J. *et al.* (2012) The Pfam protein families database. *Nucleic Acids Res.*, **40**, D290–D301.

29. Tanabe, M. and Kanehisa, M. (2012) Using the KEGG database resource. *Curr. Protoc. Bioinformatics, 2012*, doi:10.1002/0471250953.bi0112s38.

30. Uyar, B., Weatheritt, R.J., Dinkel, H., Davey, N.E. and Gibson, T.J. (2014) Proteome-wide analysis of human disease mutations in short linear motifs: neglected players in cancer? *Mol. Biosyst.*, **10**, 2626–2642.

31. Mottaz, A., David, F. P.A., Veuthey, A.-L. and Yip, Y.L. (2010) Easy retrieval of single amino-acid polymorphisms and phenotype information using SwissVar. *Bioinfromatics*, **26**, 851–852.

32. Diella, F., Haslam, N., Chica, C., Budd, A., Michael, S., Brown, N.P., Trave, G. and Gibson, T. (2008) Understanding eukaryotic linear motifs and their role in cell signaling and regulation. *Front. Biosci.*, **13**, 6580–6603.

33. MacDonald, B.T., Tamai, K. and He, X. (2009) Wnt/beta-catenin signaling: components, mechanisms, and diseases. *Dev. Cell*, **17**, 9–26.

34. Li, V.S.W., Ng, S.S., Boersema, P.J., Low, T.Y., Karthaus, W.R., Gerlach, J.P., Mohammed, S., Heck, A.J.R., Maurice, M.M., Mahmoudi, T. *et al.* (2012) Wnt signaling through inhibition of beta-catenin degradation in an intact Axin1 complex. *Cell*, **149**, 1245–1256.

35. Winston, J.T., Strack, P., Beer-Romero, P., Chu, C.Y., Elledge, S.J. and Harper, J.W. (1999) The SCFbeta-TRCP-ubiquitin ligase complex associates specifically with phosphorylated destruction motifs in IkappaBalpha and beta-catenin and stimulates IkappaBalpha ubiquitination in vitro. *Genes Dev.*, **13**, 270–283.

36. He, X., Semenov, M., Tamai, K. and Zeng, X. (2004) LDL receptor-related proteins 5 and 6 in Wnt/beta-catenin signaling: arrows point the way. *Development*, **131**, 1663–1677.

37. Takemaru, K.-I., Fischer, V. and Li, F.-Q. (2009) Fine-tuning of nuclear-catenin by Chibby and 14-3-3. *Cell Cycle*, **8**, 210–213.

38. Tompa, P., Davey, N.E., Gibson, T.J. and Babu, M.M. (2014) A million peptide motifs for the molecular biologist. *Mol. Cell*, **55**, 161–169.

39. Gibson, T.J., Dinkel, H., Van Roey, K. and Diella, F. (2015) Experimental detection of short regulatory motifs in eukaryotic proteins: tips for good practice as well as for bad. *Cell Commun. Signal*, **13**, 42.