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Protein interactome mapping in *Caenorhabditis elegans*

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Abstract

The systematic identification of all protein–protein interactions that take place in an organism (the 'interactome') is an important goal in modern biology. The nematode *Caenorhabditis elegans* was one of the first multicellular models for which a proteome-wide interactome mapping project was initiated. Most *Caenorhabditis elegans* interactome mapping efforts have utilized the yeast two-hybrid system, yielding an extensive binary interactome, while recent developments in mass spectrometry-based approaches hold great potential for further improving our understanding of protein interactome networks in a multicellular context. For example, methods like co-fractionation, proximity labeling, and tissue-specific protein purification not only identify protein–protein interactions, but have the potential to provide crucial insight into when and where interactions take place. Here we review current standards and recent improvements in protein interaction mapping in *C. elegans*.

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Yeast two hybrid, Mass spectrometry, Protein interaction, *C. elegans*.

Introduction

Since the advent of whole-genome sequencing, high-throughput (HT) approaches are increasingly important for our efforts to understand the functions of all gene products and the relationships between them. Interactions between proteins represent a large part of the interactions between macromolecules in our cells, and several HT protein–protein interaction (PPI) mapping techniques have been developed to systematically map these interactions. HT-PPI mapping technologies were initially employed to map the interactomes of single cell systems, but efforts quickly expanded to model organisms, to investigate PPI networks on a multicellular level.

The mapping of the *C. elegans* interactome has been dominated by binary interaction mapping using the yeast two-hybrid (Y2H) system (Figure 1). *C. elegans* was the first multicellular organism to have its entire genome sequenced and annotated [1]. This gave the roundworm an advantage for pioneering Y2H-based PPI mapping approaches, such as the development of complete ORFeome collections to facilitate screening efforts [2,3], and the integration of interactome maps with phenome and transcriptome data to improve the predictive capabilities of interactome networks [4–7]. The procedures and improvements developed in *C. elegans* have served as a template for similar Y2H-based interactome mapping efforts in other organisms, including human [8–11].

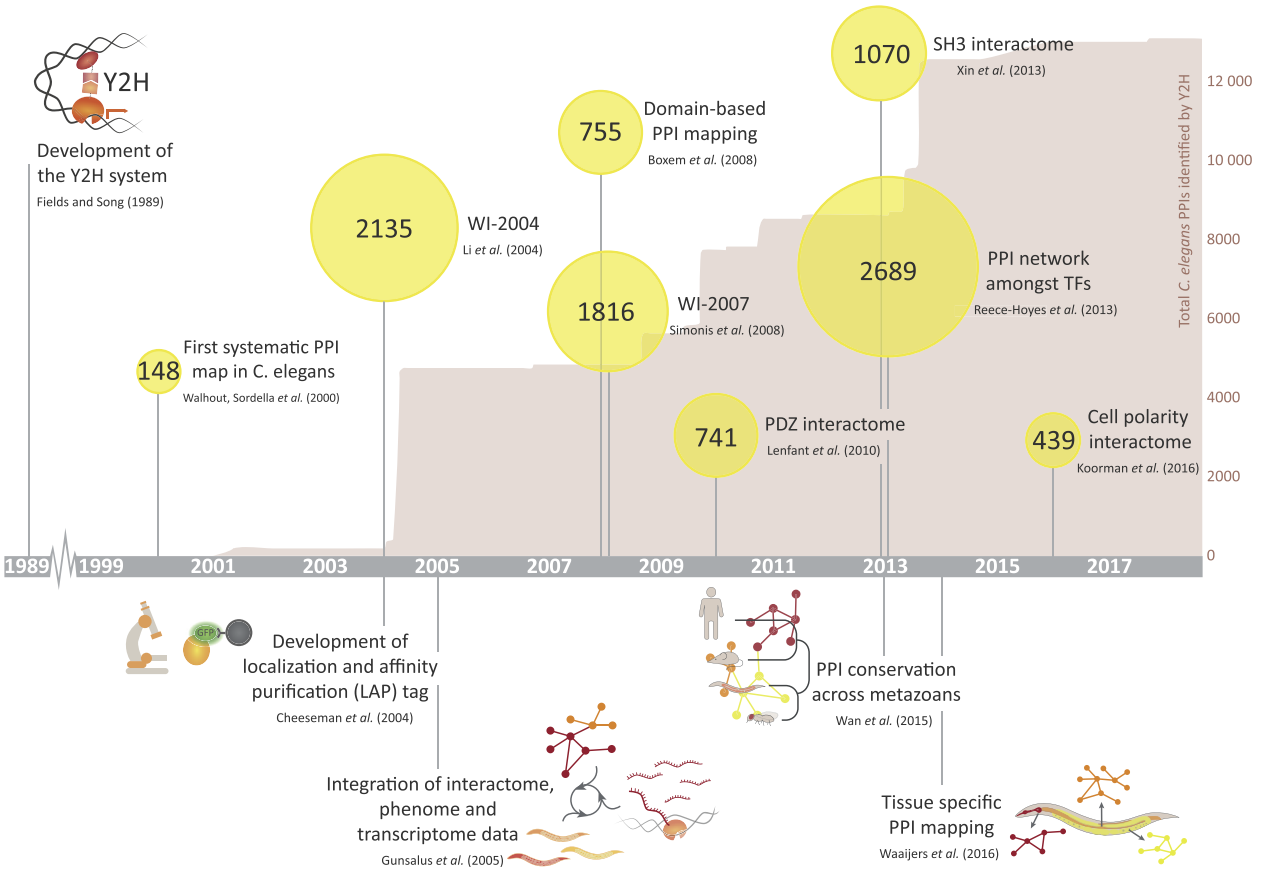
Mass spectrometry (MS)-based protein complex mapping approaches have made more modest contributions to the *C. elegans* interactome, though recently thousands of candidate *C. elegans* protein–protein associations were identified by co-fractionation (Figure 1) [12]. MS-based techniques like tissue-specific protein purification and proximity labeling enable the addition of spatiotemporal information to interactome networks, a critical step towards understanding protein–protein interactions in the context of a multicellular organism such as *C. elegans*. Here, we review the state of interactome mapping in *C. elegans*, and discuss recent developments that are likely to improve protein interaction mapping efforts in this organism in the coming years.

Binary interactome mapping – *C. elegans* as a model for genome-scale Y2H mapping

The most widely-used binary interaction assay is the Y2H system, which is based on the reconstitution of a transcription factor through binding of two hybrid proteins: one fused to a DNA binding (DB) domain, and one fused to an activation domain (AD) [13]. A major advantage of the Y2H system is its simplicity: only yeast and DNA clones need to be handled, making the Y2H system highly scalable.

The elucidation of the *C. elegans* protein interactome by Y2H took off discretely, with the mapping of a network of 148 interactions centered around proteins involved in

Figure 1



Chronological presentation of landmark publications for protein interactome mapping in *C. elegans*. Yellow circles depict number of high-confidence interactions reported in key Y2H studies. Graph represents total *C. elegans* PPIs identified by Y2H curated in IntAct.

vulval development [14]. While small in size, this study produced the first interaction network for a multicellular organism and pioneered several approaches that remain current to date. One of these was the development of Gateway recombinational cloning, which was later used to generate a genome-scale resource of *C. elegans* open reading frames (ORFs) (the ORFeome) [2,3,15]. Another was the use of matrix experiments, in which all pairwise combinations of proteins are tested. While labor intensive, the advantage of this approach is that the search space of protein pairs tested is known precisely. Matrix screens using ORFeome collections were used to map a significant fraction of the *C. elegans* binary interactome, and form the backbone of current efforts to map the human interactome by Y2H [8,9,11,16].

The first truly large-scale interaction map for *C. elegans* was published in 2004 [17]. Using 1873 metazoan-specific proteins as baits in library screens, a total of 2135 protein–protein interactions were uncovered, and the quality of the data set was verified by co-affinity purification. Together with a large scale Y2H interaction map of *Drosophila* published just weeks prior [18], this publication heralded the first proteome-scale interaction maps for multicellular organisms. In 2009, the *C. elegans* ORFeome collection, which by then covered some 10,000 genes, was used in matrix Y2H screens to expand the interactome to 3864 interactions among 2528 proteins (the Worm Interactome version 8, or WI8) [16]. The largest single addition to the *C. elegans* binary interactome since WI8 was made by Reece-Hoyes et al., who combined PPI mapping by Y2H with protein–DNA interaction (PDI) mapping by yeast one-hybrid (Y1H) in a comprehensive study of *C. elegans* transcription factor (TF) network evolution [19]. Over 2500 PPIs were uncovered between TFs or between TFs and transcriptional cofactors, and analysis of the PPI and PDI networks showed unexpectedly rapid rewiring of transcriptional networks, with even highly similar TFs often having different interaction profiles [19].

Most recent binary interactome mapping efforts by Y2H have focused on specific biological processes and novel applications of the Y2H system. An example is the use of