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Origin of RNA Polymerase II pause in eumetazoans: Insights from *Hydra*

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Abstract

Multicellular organisms have evolved sophisticated mechanisms for responding to various developmental, environmental and physical stimuli by regulating transcription. The correlation of distribution of RNA Polymerase II (RNA Pol II) with transcription is well established in higher metazoans, however genome-wide information about its distribution in early metazoans, such as *Hydra*, is virtually absent. To gain insights into RNA Pol II-mediated transcription and chromatin organization in *Hydra*, we performed chromatin immunoprecipitation (ChIP)-coupled high-throughput sequencing (ChIP-seq) for RNA Pol II and Histone H3. Strikingly, we found that *Hydra* RNA Pol II is uniformly distributed across the entire gene body, as opposed to its counterparts in bilaterians such as human and mouse. Furthermore, correlation with transcriptome data revealed that the levels of RNA Pol II correlate with the magnitude of gene expression. Strikingly, the characteristic peak of RNA Pol II pause typically observed in bilaterians at the transcription start sites (TSSs) was not observed in *Hydra*. The RNA Pol II traversing ratio in *Hydra* was found to be intermediate to yeast and bilaterians. The search for factors involved in RNA Pol II pause revealed that RNA Pol II pausing machinery was most likely acquired first in Cnidaria. However, only a small subset of genes exhibited the promoter proximal RNP Pol II pause. Interestingly, the nucleosome occupancy is highest over the subset of paused genes as compared to total *Hydra* genes, which is another indication of paused RNA Pol II at these genes. Thus, this study provides evidence for the molecular basis of RNA Pol II pause early during the evolution of multicellular organisms.

Keywords

Hydra; RNA Pol II; transcription; promoter pause; nucleosome occupancy

1 Introduction

Organization, regulation and maintenance of chromatin in different cell types are crucial for the growth and development of multicellular organisms. Understanding changes in the dynamics of chromatin and its causative molecular cues during the early evolution is essential to provide insights into the patterns of gene regulation responsible for configuring multicellular organisms (Koster *et al.* 2015). The phylogenetic position at the base of

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Eumetazoa makes *Hydra* a powerful model organism to understand the transitions that led to the evolution of gene regulation and functional genomic studies in complex multicellular organisms (Collins *et al.* 2005). *Hydra* also serves as an interesting system to study its regeneration capability (Bosch 2007), embryogenesis (Fröbisch *et al.* 2003; Genikhovich *et al.* 2006), evolution of eumetazoan specific cell types such as neurons and muscular epithelial cells (Hobmayer *et al.* 2012), aging mechanism (Tomczyk *et al.* 2015) and symbiosis (Bosch 2012). The availability of genome sequence (Chapman *et al.* 2010) and transcriptomes under different experimental conditions, along with molecular tools such as siRNA-mediated knockdown and generation of transgenic lines, makes *Hydra* amenable for molecular dissection of evolution of gene regulation. Therefore, we have chosen the basal eumetazoan *Hydra* as a model system for this study.

Previously, we reported the early evolution of the complete histone repertoire along with taxon specific histone variant (Reddy *et al.* 2017). All canonical *Hydra* histones show the conservation of amino acids important for writers and readers of epigenetic modifications. Similarly, studies in a related Cnidarian, *Hydractinia*, has also shown the conservation of canonical histones and presence of non-canonical H2B variants, which are male germ cell specific (Török *et al.* 2016). Evolution of body axis occurred in Cnidaria and the role of Wnt signalling has been shown in the organizer activity in *Hydra* (Hobmayer *et al.* 2000; Broun *et al.* 2005; Reddy *et al.* 2019). We then analysed genes differentially regulated upon the activation of Wnt signalling in *Hydra*. In this analysis, a simple *Hydra*-like body axis formation involved a major set of genes involved in various processes like cell–cell contact, cell–ECM interaction and cytoskeletal dynamics affecting tissue morphogenesis. In addition to this, interconnections to other developmental signalling pathways like hedgehog, receptor tyrosine and hormone mediated signalling pathways were observed. This study shows the evolution and adaptation of master gene regulator transcription factors into Wnt signaling network regulating a primary body axis before Bilateria–Radiata split. These players have later diverged further to give rise to A–P, D–V and Left–Right axes. Next, we studied the regulation of genes by monitoring the RNA Pol II occupancy profile and found a small set of genes involved in constitutive and stimuli responsive function. This is well correlated with the evolution of associated molecular players, such as NELF, in RNA Pol II pausing.

Until recently, one of the major rate limiting and regulated steps during transcription was thought to be RNA Pol II recruitment at transcription start sites (Adelman and Lis 2012). However, recent technical advances allowed the analysis of changes at global level, showing that RNA Pol II pausing is a hitherto underexplored and overlooked, but potentially widespread strategy for controlling gene expression (Mayer *et al.* 2017). The pausing of RNA Pol II at the promoters might help in rapid release of stalled RNA Pol II during development or environmental stimuli, that in turn facilitate an efficient and integrated response to the dynamically changing environment (Muse *et al.* 2007; Chen *et al.* 2018). Here, we mapped the sites of RNA Pol II recruitment on the *Hydra* genome and correlated it with the global transcription. Furthermore, we identified a subset of developmentally regulated genes that exhibit poised RNA Pol II in a manner characteristic of bilaterians. Thus, our study provides the evidence of an important transition in gene regulation that occurred during the emergence of complex multicellular organisms with specialized tissue types and functions.

2 Materials and methods

2.1 Hydra culture

A clonal culture of *Hydra vulgaris* Ind-Pune was maintained at 18°C using standard protocols described previously (Reddy *et al.* 2011). *Hydra* polyps were fed daily with freshly hatched *Artemia nauplii* larvae and cleaned after 6–8 h of feeding.

2.2 Antibodies

Pan Histone H3 antibody (Abcam-ab1791) and RNA Polymerase II (Pol II) mouse monoclonal antibody (7G5) were used. Both antibodies recognized respective proteins in *Hydra* (supplementary figure 1).

2.3 Chromatin immunoprecipitation (ChIP)

Two thousand *Hydra* polyps were crosslinked with 1% formaldehyde, lysed and sonicated in sonication buffer (10 mM Tris-HCl pH 7.5, 200 mM NaCl, 1% SDS, 4% NP-40, 1 mM PMSF) to obtain an average chromatin size of 300 bp. Chromatin was pre-cleared using 50 µL of a 50% protein A sepharose (GE healthcare) slurry for 1 h at 4°C with gentle inverting. Immunoprecipitations were carried out in 1 ml of ChIP buffer (20 mM Tris-HCl pH 8.0, 150 mM NaCl, 2 mM EDTA, 1% Triton-X 100). Input chromatin was obtained after preclearing, by de-crosslinking and purifying input DNA using a Qiaquick column (Qiagen) according to manufacturer's instructions. Immunoprecipitations were carried out with inverting at 4°C for 14–16 h. The samples were then incubated with 50 µL of a 50% Protein A Sepharose slurry for 3 h at 4°C with gentle inverting. ChIP samples were reverse-crosslinked and the DNA was purified using a Qiaquick column (Qiagen). Q-PCR using SYBR green was used to validate known target sites before and after sequencing.

2.4 Library preparation and next generation sequencing

Sequencing libraries were prepared using SOLiD ChIP-Seq library preparation kit according to manufacturer's instructions. DNA samples were end repaired and adapter ligated before subjecting for amplification for 12 cycles to visualise sufficiently on 2% E-gel (Thermo). DNA purification at every step was performed using Agencourt XP beads (Beckman Coulter). Average library size were estimated using the bioanalyzer 2100 high-sensitivity DNA kit (Agilent) and concentrations were measured using the Qubit fluorometer (Invitrogen). Equimolar amount of libraries were pooled and 50 bp reads were sequenced in-house using SOLiD 4.0 sequencer (Applied Biosystems).

2.5 Average profile calculations

We extracted the tag density in a 5 Kb window surrounding the TSSs and gene body using the program seqMINER, which generates heatmap as well as the profiles (Ye *et al.* 2011). The sequenced ChIP-seq reads represent only the end of each immunoprecipitated fragments instead of the precise protein–DNA binding sites. To illustrate the entire DNA fragment, basically before analysis, 3' end of each ChIP-seq read was extended to 200 bp in the direction of the reads. For average gene profiles, genes (+/–5000 bp from binding site) were divided in 100 bins of length relative to the gene length. Moreover, 10 equally sized (50 bp)

bins were created on the 5' and 3' of the gene and ChIP-seq densities were collected for each dataset in each bin. The average of enrichments over input in each bin was plotted for each dataset.

2.6 Data source and analysis

ChIP-seq datasets for RNA Pol II were downloaded from the public data bank Gene Expression Omnibus (<http://www.ncbi.nlm.nih.gov/gds>) under the accession numbers GSM1160310 (*Drosophila*), GSM840279 (Human), GSM1018217 (Yeast) and GSM307623 (Mouse). The scatter plots, k-means clustering and average gene profiles were created using seqMiner (Ye *et al.* 2011). Box plots and correlation analysis were produced using R software (<http://r-project.org/>). Integrative Genomics Viewer (IGV) was used for data visualization. The sequencing data we have generated is deposited in the GEO database under the accession number GSE63414. The number of mapped reads for each data set is provided in supplementary table 1. The primers used for ChIP-qPCR validation are provided in supplementary table 2.

2.7 Phylogenetic analysis

NELF (negative elongation factor) homologues were initially identified by BLAST-P similarity search performed using human counterparts as query from representative model organisms (supplementary table S3). A detailed phylogenetic analysis was carried out using these sequences. Here, initial alignment was carried out using MUSCLE (default parameters) and alignments were trimmed using trimAl (auto mode), which are implemented on an online server (<https://ngphylogeny.fr>) (Lemoine *et al.* 2019). An unrooted phylogenetic tree was computed using randomized accelerated maximum likelihood (RAxML-HPC v.8 on XSEDE) implemented in CIPRES Science Gateway (Miller *et al.* 2012). Here, PROTCAT substitution model with DAHOFF substitution matrix was used with 1000 iterations of rapid bootstrap analysis. The phylogenetic tree was visualized and annotated in iTOL and branch support values above 30 were displayed on branches (Letunic and Bork 2016).

3 Results

3.1 RNA Pol II pause immediately upstream of transcription start sites (TSSs) is not evident in Hydra

The distribution and the correlation of RNA Polymerase II (RNA Pol II) occupancy with transcription are well established in bilaterians, however nothing is known about its distribution in basal eumetazoans such as *Hydra*. To gain insights into the RNA Pol II profile and transcription machinery in *Hydra*, we performed chromatin immunoprecipitation (ChIP)-coupled highthroughput sequencing (ChIP-seq) for RNA Pol II using commercially available antibody (supplementary figure 1). Interestingly, we found that *Hydra* RNA Pol II is uniformly distributed across the entire gene body (figure 1A) as opposed to its counterparts in bilaterians such as human and mouse (Adelman and Lis 2012). Further, to contrast our observation with other metazoans, we calculated the density of RNA Pol II in the gene body of five eukaryotic model systems: human, mouse, drosophila, yeast and *Hydra*. Our analysis suggests highest RNA Pol II pausing at the TSSs in mouse and human

followed by *Drosophila* and no RNA Pol II pausing in yeast. Interestingly, the RNA Pol II pause in *Hydra* was minimal, and the distribution of RNA Pol II in gene body was maximum (figure 1A). Representative examples of uniform distribution of *Hydra* RNA Pol II genome browser tracks are shown in figure 1B. The uniform distribution of RNA Pol II obtained in ChIP-sequencing was also validated by ChIP-qPCR on randomly selected genomic loci enriched with RNA Pol II (supplementary figure 2). Furthermore, uniform distribution of *Hydra* RNA Pol II was confirmed by correlating ChIP-seq enrichments in a second independent replicate (Pearson correlation coefficient = 0.98, supplementary figure 3). The uniform distribution of *Hydra* RNA Pol II prompted us to explore the correlative relationship between the distribution of RNA Pol II over the gene body and the levels of transcription of the corresponding genes. The density of RNA Pol II was calculated over 500 genes with high, medium and very low expression levels, each, and plotted as an average gene profile. Interestingly, the levels of RNA Pol II correlate with the magnitude of gene expression. However, the characteristic peak of RNA Pol II pause at the TSSs found in bilaterians was not observed in *Hydra* RNA Pol II (figure 1C). Taken together, our data suggests that though *Hydra* RNA Pol II occupancy correlates with gene expression, it does not exhibit the characteristic RNA Pol II pause like other higher eukaryotic systems.

3.2 Hydra exhibits slowest RNA Pol II traveling ratio

In bilaterian model organisms, differential gene expression depends on the regulation of transcription elongation via the release of RNA Pol II from the promoter (Levine 2011; Kwak and Lis 2013). Absence of promoter RNA Pol II in *Hydra* prompted us to calculate the RNA Pol II traveling ratio or 'pausing ratio', which quantifies the relative amount of RNA Pol II binding near the promoter versus at the gene body (Rahl *et al.* 2010). Decrease in RNA Pol II travelling ratio is an indication of clearance of promoter or transcription elongation inhibition (Wade and Struhl 2008). We then calculated the RNA Pol II traveling ratio for human, mouse, *Drosophila* and yeast, along with *Hydra* (figure 2). A higher traveling ratio value indicates a higher degree of pausing (Rahl *et al.* 2010). Interestingly, the RNA Pol II travelling ratio was found to be the lowest in *Hydra*, reinforcing the observation that it presumably lacks the RNA Pol II pause and indicating a different mode of RNA Pol II mediated transcriptional initiation and regulatory mechanism in *Hydra*. Interestingly, the global distribution of RNA Pol II in *Hydra* is intermediary to yeast and bilaterians (figure 1A). However, the traveling ratio is lower than that of yeast (figure 2). Thus, a multicellular organism such as *Hydra*, which exhibits differential gene expression in spatio-temporal manner according to external or internal signals, characteristically lacks an important developmental checkpoint present in higher metazoan; the RNA Pol II pause.

3.3 Evolution of NELF family members

Promoter-proximal pausing of RNA Pol II is a post-initiation regulatory event and we wondered if *Hydra*, which lacks the promoter-proximal pause, uses novel mechanisms of transcriptional initiation/elongation. It has been shown that a multisubunit factor (Negative elongation factor – NELF) in association with DRB sensitivity-inducing factor (DSIF) contributes to the transcriptional pausing of RNA Pol II (Narita *et al.* 2003). The genes regulated by promoter-proximal pause are shown to have binding of pause factor, the negative elongation factor (NELF) downstream of the TSS (Wada *et al.* 1998). The NELF

complex consists of four distinct polypeptides namely NELF-A, NELF-B, NELF-C/D and NELF-E (Yamaguchi *et al.* 1999, 2001; Narita *et al.* 2003). Among these, NELF-E has the RNA binding domain and plays a critical role in NELF activity (Yamaguchi *et al.* 2002). Human homologues of these components were identified in mouse and *Drosophila* and are not present in *Caenorhabditis elegans* and *Saccharomyces cerevisiae*. We screened for homologues of these components in our in-house transcriptome assembly generated from *Hydra vulgaris* Ind-Pune using protein blast similarity search (supplementary table 3) and performed a maximum likelihood phylogenetic analysis after performing multiple sequence alignment (supplementary figure 4) to ascertain their homology to their bilaterian counterparts (figure 3A). Surprisingly, we found that the genes for all the NELF factors necessary for RNA Pol II pause were coded by *Hydra*. A comparative phylogenetic analysis suggest that NELF-A, B and C/D occurred before the divergence of sponges and the complete NELF repertoire evolved before the divergence of Cnidaria and Bilateria. Interestingly, putative homologues are not present in *Schmidtea mediterranea* (*Platyhelminthes*) and *Caenorhabditis elegans* (*Nemathelminthes*) suggesting intermittent loss of NELF members in these lineages (figure 3). This suggests that members of the complete NELF complex are present in *Hydra* and might be involved in the RNA Pol II pause, similar to the bilaterians.

3.4 A select subset of genes exhibit RNA Pol II pause in Hydra

The absence of global RNA Pol II pause but presence of all the subunits of pause factor, NELF prompted us to re-examine the RNA Pol II pause in *Hydra*. We screened for individual genes, which exhibit RNA Pol II pause at the TSSs. Interestingly, such an exercise yielded a set of 235 genes, wherein significant RNA Pol II pause at TSSs was observed (figure 4A). To understand the role of RNA Pol II pause in *Hydra*, we compared the expression level of RNA Pol II paused genes as opposed to global transcription. Here, we found that RNA Pol II paused genes in *Hydra* are highly expressed as compared to the global transcription (figure 4B). Gene ontology (GO) analysis of RNA Pol II paused genes demonstrated that these genes are mostly associated with functions specific to multicellular organisms, localization and pattern formation, developmental processes and response to stimulus (figure 4C). Thus, the pausing of RNA Pol II is a plausible mechanism to fine-tune gene expression and to potentiate genes for further and/or future activation on a select subset of genes, which are important for stimuli-dependent or development-related functions.

3.5 Strongly positioned +1 nucleosome facilitates RNA Pol II pause in Hydra

Nucleosomes form barriers against RNA Pol II elongation and paused genes maintain strong nucleosome signatures near transcription start sites (Jimeno-González *et al.* 2015). To further understand if the differential transcriptional regulation can be a function of distribution of nucleosomes in *Hydra*, we performed histone H3 ChIP-sequencing for determining nucleosome positioning in *Hydra*. Mapping of the ChIP-seq reads on the *Hydra* genome revealed that the nucleosomes are highly dynamic and unstable in *Hydra* as compared to yeast and the +1 nucleosome flanking the promoter is located at a preferred position along with nucleosome free region at TSSs (supplementary figure 5). Furthermore, the nucleosome occupancy is highest over the paused genes as compared to the total *Hydra* genes (figure 5), which is another indication of paused RNA Pol II at these genes. Taken

together, our results indicate that the strongly positioned +1 nucleosome might be an important factor in facilitating promoter-proximal pausing of RNA Pol II to fine tune gene expression of important stimuli-dependent and developmentally regulated genes.

4 Discussion

Transcription is not merely the expression of the information encoded by the genome. Rather, it is a multilayered procedure that ultimately integrates cellular demands in terms of gene expression. Living organisms are critically dependent on their fixed inherited genetic constitution and on the changing environment to which they are exposed to and typically respond to the internal or external cues through tightly controlled modulation of transcriptional responses. Thus, transcription programmes ultimately define the identity, function and fate of every cell of an organism. Multicellular organisms have evolved sophisticated mechanisms for responding to various developmental, environmental and physical stimuli by regulating transcription. In a multicellular organism such as *Hydra*, the mechanism of RNA Pol II mediated transcriptional regulation appears to be in between that of yeast (unicellular eukaryote) and multicellular bilaterians. Our observations suggest that genes with high levels of paused RNA Pol II inherently favor the formation of nucleosomes over the promoter, establishing an active competition between RNA Pol II and nucleosomes for promoter occupancy (Gilchrist *et al.* 2010). The intrinsically repressive chromatin structure prevents aberrant expression of paused genes, which are often component of highly regulated pathways (Liu *et al.* 2015; Shao and Zeitlinger 2017). Our analysis suggests that the RNA Pol II pausing machinery was possibly first acquired in *Hydra*; however, it was restricted to a small subset of genes that were required for stimuli-dependent and developmentally regulated genes. Presence of all NELF complex members in *Hydra* indicates their role in observed RNA Pol II pausing. This in turn suggests that the pausing of RNA Pol II at the select TSSs in *Hydra* is a mechanism for tuning the expression of active genes and presumably maintaining them in poised state for future burst in gene expression.

Previous studies in mammals and *Drosophila* have suggested the role of promoter sequence elements such as presence of GAGA motif, downstream promoter element (DPE), pause button (PB) and correlate with the higher GC content (Amir-Zilberstein *et al.* 2007; Hendrix *et al.* 2008; Gilchrist *et al.* 2010). These findings suggest that further analysis on the evolution of these elements might shed light on intrinsic properties of the promoters in regulation of RNA Pol II pause. This study suggests eumetazoan genes started ‘learning’ to pause and facilitate differential regulation required for development of multicellular organisms before the divergence of Bilateria and Radiata. Studies in *Nematostella* based on genome-wide histone marks have shown that existence of conserved eumetazoan enhancer mediated gene regulatory mechanism (Schwaiger *et al.* 2014). In light of the current study and other reports, cnidarian models serve as excellent model organisms to understand the evolutionary transition of gene regulation observed in complex multi-cellular organisms.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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References

- Adelman K, Lis JT. Promoter-proximal pausing of RNA polymerase II: emerging roles in metazoans. *Nat Rev Genet.* 2012; 13:720–731. [PubMed: 22986266]
- Amir-Zilberstein L, Ainbinder E, Toubé L, Yamaguchi Y, Handa H, Dikstein R. Differential regulation of NF- κ by elongation factors is determined by core promoter type. *Mol Cell Biol.* 2007; 27:5246–5259. [PubMed: 17502349]
- Bosch TC. Why polyps regenerate and we don't: towards a cellular and molecular framework for *Hydra* regeneration. *Dev Biol.* 2007; 303:421–433. [PubMed: 17234176]
- Bosch TC. What *Hydra* has to say about the role and origin of symbiotic interactions. *Biol Bull.* 2012; 223:78–84. [PubMed: 22983034]
- Broun M, Gee L, Reinhardt B, Bode HR. Formation of the head organizer in hydra involves the canonical Wnt pathway. *Development.* 2005; 132:2907–2916. [PubMed: 15930119]
- Chapman JA, Kirkness EF, Simakov O, Hampson SE, Mitros T, Weinmaier T, Rattei T, Balasubramanian PG, Borman J, Busam D. The dynamic genome of *Hydra*. *Nature.* 2010; 464:592–596. [PubMed: 20228792]
- Chen FX, Smith ER, Shilatifard A. Born to run: control of transcription elongation by RNA polymerase II. *Nat Rev Mol Cell Biol.* 2018; 19:464–478. [PubMed: 29740129]
- Collins AG, Cartwright P, McFadden CS, Schierwater B. Phylogenetic context and basal metazoan model systems. *Integr Comp Biol.* 2005; 45:585–594. [PubMed: 21676805]
- Fröbisch AC, Genikhovich G, Kürn U, Anton-Erxleben F, Bosch TC. Expression of developmental genes during early embryogenesis of *Hydra*. *Dev Genes Evol.* 2003; 213:445–455. [PubMed: 12883882]
- Genikhovich G, Kürn U, Hemmrich G, Bosch TC. Discovery of genes expressed in *Hydra* embryogenesis. *Dev Biol.* 2006; 289:466–481. [PubMed: 16337937]
- Gilchrist DA, Dos Santos G, Fargo DC, Xie B, Gao Y, Li L, Adelman K. Pausing of RNA polymerase II disrupts DNA-specified nucleosome organization to enable precise gene regulation. *Cell.* 2010; 143:540–551. [PubMed: 21074046]
- Hendrix DA, Hong J-W, Zeitlinger J, Rokhsar DS, Levine MS. Promoter elements associated with RNA Pol II stalling in the *Drosophila* embryo. *Proc Natl Acad Sci U S A.* 2008; 105:7762–7767. [PubMed: 18505835]
- Hobmayer B, Jenewein M, Eder D, Eder M-K, Glasauer S, Gufler S, Hartl M, Salvenmoser W. Stemness in *Hydra*—a current perspective. *Int J Dev Biol.* 2012; 56:509–517. [PubMed: 22689357]
- Hobmayer B, Rentzsch F, Kuhn K, Happel CM, von Laue CC, Snyder P, Rothbacher U, Holstein TW. WNT signalling molecules act in axis formation in the diploblastic metazoan *Hydra*. *Nature.* 2000; 407:186–189. [PubMed: 11001056]
- Jimeno-González S, Payán-Bravo L, Muñoz-Cabello AM, Guijo M, Gutierrez G, Prado F, Reyes JC. Defective histone supply causes changes in RNA polymerase II elongation rate and cotranscriptional pre-mRNA splicing. *Proc Natl Acad Sci U S A.* 2015; 112:14840–14845. [PubMed: 26578803]
- Koster MJ, Snel B, Timmers HT. Genesis of chromatin and transcription dynamics in the origin of species. *Cell.* 2015; 161:724–736. [PubMed: 25957681]
- Kwak H, Lis JT. Control of transcriptional elongation. *Annu Rev Genet.* 2013; 47:483–508. [PubMed: 24050178]

- Lemoine F, Correia D, Lefort V, Doppelt-Azeroual O, Mareuil F, Cohen-Boulakia S, Gascuel O. NGPhylogeny. fr: new generation phylogenetic services for non-specialists. *Nucleic Acids Res.* 2019; 47:W260–W265. [PubMed: 31028399]
- Letunic I, Bork P. Interactive tree of life (iTOL) v3: an online tool for the display and annotation of phylogenetic and other trees. *Nucleic Acids Res.* 2016; 44:W242–W245. [PubMed: 27095192]
- Levine M. Paused RNA polymerase II as a developmental checkpoint. *Cell.* 2011; 145:502–511. [PubMed: 21565610]
- Liu X, Kraus WL, Bai X. Ready, pause, go: regulation of RNA polymerase II pausing and release by cellular signaling pathways. *Trends Biochem Sci.* 2015; 40:516–525. [PubMed: 26254229]
- Mayer A, Landry HM, Churchman LS. Pause & go: from the discovery of RNA polymerase pausing to its functional implications. *Curr Opin Cell Biol.* 2017; 46:72–80. [PubMed: 28363125]
- Miller, MA; Pfeiffer, W; Schwartz, T. The CIPRES science gateway: enabling high-impact science for phylogenetics researchers with limited resources. Paper presented at: Proceedings of the 1st Conference of the Extreme Science and Engineering Discovery Environment: Bridging from the eXtreme to the campus and beyond (ACM); 2012.
- Muse GW, Gilchrist DA, Nechaev S, Shah R, Parker JS, Grissom SF, Zeitlinger J, Adelman K. RNA polymerase is poised for activation across the genome. *Nat Genet.* 2007; 39:1507–1511. [PubMed: 17994021]
- Narita T, Yamaguchi Y, Yano K, Sugimoto S, Chanarat S, Wada T, Kim D-K, Hasegawa J, Omori M, Inukai N. Human transcription elongation factor NELF: identification of novel subunits and reconstitution of the functionally active complex. *Mol Cell Biol.* 2003; 23:1863–1873. [PubMed: 12612062]
- Rahl PB, Lin CY, Seila AC, Flynn RA, McCuine S, Burge CB, Sharp PA, Young RA. c-Myc regulates transcriptional pause release. *Cell.* 2010; 141:432–445. [PubMed: 20434984]
- Reddy PC, Barve A, Ghaskadbi S. Description and phylogenetic characterization of common *Hydra* from India. *Curr Sci.* 2011; 101:736–738.
- Reddy PC, Gungi A, Ubhe S, Pradhan SJ, Kolte A, Galande S. Molecular signature of an ancient organizer regulated by Wnt/ β -catenin signalling during primary body axis patterning in *Hydra*. *Commun Biol.* 2019; 2:1–11. [PubMed: 30740537]
- Reddy PC, Ubhe S, Sirwani N, Lohokare R, Galande S. Rapid divergence of histones in Hydrozoa (Cnidaria) and evolution of a novel histone involved in DNA damage response in *Hydra*. *Zool (Jena).* 2017; 123:53–63.
- Schwaiger M, Schönauder A, Rendeiro AF, Pribitzer C, Schauer A, Gilles AF, Schinko JB, Renfer E, Fredman D, Technau U. Evolutionary conservation of the eumetazoan gene regulatory landscape. *Genome Res.* 2014; 24:639–650. [PubMed: 24642862]
- Shao W, Zeitlinger J. Paused RNA polymerase II inhibits new transcriptional initiation. *Nat Genet.* 2017; 49:1045–1051. [PubMed: 28504701]
- Tomczyk S, Fischer K, Austad S, Galliot B. *Hydra*, a powerful model for aging studies. *Invertebr Reprod Dev.* 2015; 59:11–16. [PubMed: 26120246]
- Török A, Schiffer PH, Schnitzler CE, Ford K, Mullikin JC, Baxevanis AD, Bacic A, Frank U, Gornik SG. The cnidarian *Hydractinia echinata* employs canonical and highly adapted histones to pack its DNA. *Epigenet Chromatin.* 2016; 9:36.
- Wade JT, Struhl K. The transition from transcriptional initiation to elongation. *Curr Opin Genet Dev.* 2008; 18:130–136. [PubMed: 18282700]
- Wada T, Takagi T, Yamaguchi Y, Ferdous A, Imai T, Hirose S, Sugimoto S, Yano K, Hartzog GA, Winston F. DSIF, a novel transcription elongation factor that regulates RNA polymerase II processivity, is composed of human Spt4 and Spt5 homologs. *Genes Dev.* 1998; 12:343–356. [PubMed: 9450929]
- Yamaguchi Y, Deléhouzée S, Handa H. HIV and hepatitis delta virus: evolution takes different paths to relieve blocks in transcriptional elongation. *Microb Infect.* 2002; 4:1169–1175.
- Yamaguchi Y, Filipovska J, Yano K, Furuya A, Inukai N, Narita T, Wada T, Sugimoto S, Konarska MM, Handa H. Stimulation of RNA polymerase II elongation by hepatitis delta antigen. *Science.* 2001; 293:124–127. [PubMed: 11387440]

- Yamaguchi Y, Takagi T, Wada T, Yano K, Furuya A, Sugimoto S, Hasegawa J, Handa H. NELF, a multisubunit complex containing RD, cooperates with DSIF to repress RNA polymerase II elongation. *Cell*. 1999; 97:41–51. [PubMed: 10199401]
- Ye T, Krebs AR, Choukrallah M-A, Keime C, Plewniak F, Davidson I, Tora L. seqMINER: an integrated ChIP-seq data interpretation platform. *Nucleic Acids Res*. 2011; 39:e35–e35. [PubMed: 21177645]

Figure 1.

Global RNA Pol II pausing is not evident in *Hydra*. *Hydra* RNA Pol II is uniformly distributed across the gene body. **(A)** Genome browser tracks of representative examples of loci showing uniform distribution of *Hydra* RNA Pol II in gene body. **(B)** Mean tag density of *Hydra* RNA Pol II calculated for whole gene and plotted for high, medium and least expressed genes (500 each). Levels of *Hydra* RNA Pol II correlate with magnitude of gene expression. **(C)** Normalized ChIP-seq enrichment for RNA Pol II from *Drosophila*, human, mouse, *S. cerevisiae* and *Hydra*. RNA Pol II pause was observed in mouse, human

and *Drosophila*. RNA Pol II pause in *Hydra* was minimal (small peak at TSS as compared to other organisms) and the distribution of RNA Pol II in gene body was maximum.

Figure 2.

Hydra RNA Pol II travelling ratio. Comparative RNA Pol II traveling ratio. RNA Pol II travelling ratio was calculated for genes from *Drosophila*, human, mouse, *S. cerevisiae* and *Hydra*. RNA Pol II travelling ratio was lowest in *Hydra* in all the examined species.

Figure 3.

Evolution of NELF family members. Reported and putative NELF protein sequences were extracted from representative phyla and their phylogenetic analyses were performed using maximum likelihood method and their occurrence was summarized. **(A)** Phylogenetic tree of four classes of NELF members. All four classes of NELF members are shaded in distinct colours. NELF homologues identified in *Hydra vulgaris* Ind-Pune are highlighted in red colour font. Numbers on branch points denote bootstrap support values. **(B)** Summary table displaying the presence of NELF members in representative model organisms. Empty circle – absence; filled circle – presence and red filled circles represent homologues found in *Hydra*.

Figure 4.

A select subset of genes exhibit RNA Pol II pausing in *Hydra*. **(A)** Average ChIP-seq profile of RNA Pol II occupancy over a subset of 235 promoters identified having paused RNA Pol II in *Hydra*. **(B)** RNA Pol II paused genes in *Hydra* are highly expressed as compared to the total transcripts levels. **(C)** Gene ontology analysis of RNA Pol II paused genes suggests enrichment of the pathways associated with gene expression regulation and translation.

Figure 5.

+1 Nucleosome is tightly positioned on transcription start sites (TSSs) of RNA Pol II paused genes in *Hydra*. +1 nucleosome position was analysed on all the genes and RNA Pol II paused genes from *Hydra*. RNA Pol II paused genes exhibit highest +1 nucleosome occupancy.