or P6.p descendants (Figure 1B). Once the integrity of the physical barrier between the AC and the vulva is compromised, the AC starts invading by extending a finger-like projection between the central vulval cells (Figures 1C–1F). The invasion starts before the central vulval cells complete their divisions, and when vulval proliferation is blocked by hydroxyurea, AC invasion is only delayed, confirming that the invaded vulval cells induce AC invasion independently of the cell lineage (Sherwood and Sternberg, 2003). This is consistent with the recent finding that vulval cells continue differentiating even if they fail to divide (Shemer and Podbilewicz, 2002). Probably, if the AC failed to invade, then the vulva would have no hole in its apex and no connection would form to the uterus. Removal of the vulval inducers by microsurgery or mutations results in the formation of long directed AG-derived filopodia that find and invade vulval tissue (Figure 1H). Thus, the descendants of P6.p or P6.p-like cells attract and induce AC invasion at a distance. However, in 24% of the operated worms and in 20% of the mutant animals that lack vulval inducer cells there was invasion of nonvulval epidermal cells, suggesting that there is also a vulva-independent signal. This abnormal AC behavior may be the result of a cell-autonomous activity or it may reflect a weaker signal from the epidermis or muscles. This second signal is proposed to be independent of the strong diffusible signal derived from the P6.p descendants (Sherwood and Sternberg, 2003). An alternative explanation is that the putative nonvulval cells may be able to send the primary inducing cue even when they appear morphologically to be epidermal.

Sherwood and Sternberg accomplish a fascinating description of the spatial and temporal behavior of the AC invader that will allow the answering of the following questions:

- What is the molecular nature of the cues that attract and induce AC invasion?
- How is the competence of the AC to respond to cell invasion cues regulated?
- How are the gonadal and vulval basement membranes degraded?
- What are the receptors and ligands responsible for the AC-matrix interactions?

How does the AC interact with the vulval P6.p cell and its descendants?

Is anchor cell fusion essential to end the invasion and to form the connection?

Genetic approaches have identified mutants with defects in the connection of gonad (Cog) to the vulva including cog-1, cog-2, lin-11, and lin-29 (Hanna-Rose and Han, 1999; Palmer et al., 2002). In addition, morphogenesis mutants with everted (Evi), squashed (Sqv), or protruding (Pvi) vulvae may identify the armaments or molecular signals and motives used by the invader and the invaded cells (Eisenmann and Kim, 2000; Herman et al., 1999; Seydoux et al., 1993). Sherwood and Sternberg have established a new model system to visualize and manipulate developmental cell invasion in vivo (Sherwood and Sternberg, 2003). As in the case of apoptosis, future cellular, molecular, and genetic studies on the invasion of the anchor cell will certainly connect not only the nematode vulva and uterus but will also reveal novel universal cell invasive mechanisms used by good and bad invaders and conserved from worms to humans.

Benjamin Podbilewicz
Department of Biology
Technion-Israel Institute of Technology
Haifa, 32000
Israel

Selected Reading

Getting (Chromosomes) Loaded—
A New Role for Timeless

A recent study in C. elegans reveals an unanticipated link between sister chromatid cohesion and the TIM-1 protein, a homolog of the Drosophila circadian rhythm protein TIMELESS. The phenotypes of tim-1 mutants suggest that cohesin subunits load onto chromosomes in a stepwise manner. Whether TIM-1 is also involved in circadian rhythms is discussed.

It is rare that research fields as diverse as chromosome segregation and circadian rhythms converge, but a recent study published in Nature stumbled upon a possible connection between the two processes. In an attempt to learn more about sister chromatid cohesion in C. elegans, Chan et al. (2003) discovered that the TIM-1 protein, which is homologous to the Drosophila circadian rhythm protein TIMELESS, physically associates with the cohesin complex that links the two sister chromatids. In the past few years, sister chromatid cohesion has emerged as a crucial component of many processes, including chromosome segregation, recombination, and repair (reviewed in Jessberger, 2002). In meiosis, the cohesin complex is required for proper...
chromosome segregation, as well as for the formation of the synaptonemal complex (SC), the structure that holds the two homologous chromosomes together. Whether cohesion only dictates proper chromosome structure needed for SC formation, or whether it is an integral part of the SC itself is currently unknown, although direct interactions between cohesin subunits and SC components support the latter (Eijpe et al., 2003, and references therein). The cohesin holocomplex is composed of four conserved subunits: Scc1/Mcd1/Rad21 (henceforth Scc1), Scc3, and two subunits of the Smc (structural maintenance of chromosomes) family, Smc1 and Smc3 (Jessberger, 2002). In meiosis, Scc1 is replaced with a meiosis-specific variant called Rec8. Recent studies suggest that the cohesin complex forms a ring, both in solution and when associated with chromosomes (Gruber et al., 2003). If, as proposed by the current model, the cohesin ring embraces the DNA, this raises the interesting question of how the ring is loaded onto chromosomes.

A connection between TIM-1 and sister chromatid cohesion was established when TIM-1 was found to copurify with cohesin isolated from C. elegans cell lysates (Chan et al., 2003). The binding of TIM-1 to the complex was weaker than that of the cohesin subunits to each other. Inactivation of tim-1 by RNAi led to an embryonic lethal phenotype. Analysis of the meiotic germ line revealed that in early meiosis, when homologs should be tightly paired, TIM-1 inactivation caused unpairing of homologous chromosomes and premature separation. What could explain this meiotic defect? Failure of homologs to pair could be due to SC abnormalities, and indeed, in tim-1 mutants, the SC was disrupted, a phenotype previously described for the inactivation of the cohesin subunit REC-8 (Pasierbek et al., 2001). Given the physical interaction between TIM-1 and the cohesin complex, and the fact that tim-1 mutants exhibit cohesion defects, it was possible that the underlying cause of the homolog pairing and SC defects in tim-1 mutants was aberrant cohesion. This prompted Chan et al. to examine the localization of different cohesin subunits in the absence of TIM-1 function. Interestingly, TIM-1 inactivation led to REC-8 mislocalization, whereas the association of the SMC subunits with chromosomes was not affected. This observation indicates that the nematode cohesin SMC subunits can associate with DNA when not part of the intact cohesin complex. Consistent with this possibility, RNAi of rec-8 did not disrupt SMC-1 or SMC-3 chromosome association (Chan et al., 2003).

It will be interesting to determine the reciprocal situation, whether REC-8 and SCC-3 can associate with chromatin in the absence of SMC-1 and SMC-3.

What might be the function of TIM-1? The observation that in the absence of TIM-1 only the SMC subunits associate with chromatin suggests that TIM-1 is needed to promote the stable binding of REC-8 and SCC-3 to the other cohesin subunits. Thus, the findings of the present study suggest that cohesin ring assembly occurs in a stepwise manner: the SMC subunits may associate with chromosomes independently of REC-8 and SCC-3, and TIM-1 may be needed to promote ring formation by enabling the binding of REC-8 and SCC-3 to chromatin-bound SMC subunits. Are there TIM-1-like functions in other organisms? Work in budding and fission yeast uncovered several proteins that are not integral parts of the cohesin complex, yet are required for its proper function. Of these, Scc2/Mis4 and Pds5 have properties that partially resemble those of TIM-1. Scc2/Mis4, along with Scc4, are required for loading cohesin onto chromosomes (Ciosk et al., 2000). Significantly, in the absence of Scc2, the binding of Scc1 and Scc3 to chromosomes is abolished whereas that of Smc1 is only reduced (Ciosk et al., 2000), resembling the phenotype generated by TIM-1 inactivation. Pds5 is essential for establishing and maintaining sister chromatid cohesion, but it is only loosely associated with the cohesin complex, as is TIM-1 (Hartman et al., 2000; Panizza et al., 2000). Tim-1, Scc2, and Pds5 all contain HEAT/Armadillo repeat elements (Panizza et al., 2000) that are thought to facilitate protein-protein interactions. Future studies should elucidate the molecular functions of TIM-1 and how it relates to those of Scc2 and Pds5.

At this point it is not clear whether TIM-1 plays a role in circadian rhythms. The timeless gene in Drosophila is essential for circadian rhythms, but not essential for viability. In contrast, the present study in C. elegans and recent studies in mouse (Gotter et al., 2000) revealed that mutations in their respective timeless orthologs are embryonic lethal. A second timeless paralog in Drosophila was identified, called tim-2/timeout (Benna et al., 2000; Gotter et al., 2000). A mutant phenotype for timeout has not been reported to date. Importantly, at the sequence level, the mouse and nematode timeless genes are more related to timeout than they are to Drosophila timeless. Chan et al. noted that nematode TIM-1, mouse Tim-1, and Drosophila TIMEOUT all have a region of contiguous HEAT/Armadillo repeats, whereas the Drosophila TIMELESS has a small insertion in its HEAT/Armadillo repeats where it interacts with the PERIOD protein. C. elegans does have a PERIOD protein, LIN-42 (Jeon et al., 1999), and circadian rhythms in this organism have been observed (Kippert et al., 2002; Saigusa et al., 2002), but whether LIN-42 and TIM-1 interact and whether they play a role in these circadian rhythms have not been examined. Thus, it is possible that the conserved function of the tim-1/timeout gene family involves the regulation of sister chromosome cohesion, a prediction that awaits further studies. Do TIM-1 proteins double as cohesin regulatory factors and circadian rhythm factors? Only time(less) will tell.

Andy Golden1 and Orna Cohen-Fix2
1Laboratory of Biochemistry and Genetics
2Laboratory of Molecular and Cellular Biology
NIDDK
NIH
Bethesda, Maryland 20892

Selected Reading


