

Cell Cycle Features:

Regulation of MEF2s by chaperone-mediated autophagy

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Chaperone-mediated autophagy (CMA) is a process whereby specific cytoplasmic substrates are targeted to lysosomes for degradation.¹ Autophagy has been implicated in playing important roles in various cellular processes including aging and neurodegeneration.² But no mechanisms have been identified to link CMA directly to neuronal survival machinery. Recent studies by us reveal such a mechanism,³ which involves CMA-mediated targeting and degradation of transcription factor myocyte enhancer factor D (MEF2D), a protein known to promote neuronal survival, by lysosomes. We showed that the level of MEF2D in neurons is regulated by CMA under basal conditions. Disruption of this process by α -synuclein leads to accumulation of non-functional MEF2D, which may underlie the pathogenic process in Parkinson's disease.

MEF2D is one of the four isoforms of MEF2s identified in mammalian cells, several of which are expressed in neurons and shown to play a role in neuronal survival. Since different MEF2 isoforms form either homo- or heterodimers to bind target DNA, it is conceivable that targeting MEF2D by CMA may affect the function of other MEF2 isoforms dependent on the MEF2 composition in a cell. Although their amino acid sequence at the C terminus differs considerably, the four MEF2 isoforms share a highly homologous N terminal region, which contains the motifs required for the interaction between MEF2D and Hsc70. Our studies showed that the N terminus of MEF2D alone is not only necessary for its binding to Hsc70 and to lysosomes but also sufficient to mediate these interactions.³ This raises an interesting possibility that other MEF2 isoforms may also interact with Hsc70 and be regulated by CMA. The four MEF2 isoforms are expressed in both developing and mature neurons. The temporal and spatial patterns of expression of different MEF2 isoforms in the nervous system are quite complex and what regulates their expression is largely unknown. Functionally, MEF2s have been implicated in the regulation of other neuronal processes such as differentiation and synaptic plasticity in addition to neuronal survival. In this regard, macroautophagy, a form of autophagy which employs a distinct molecular mechanism from CMA, has been postulated to play roles in neurodevelopment and plasticity. Therefore, it would be interesting to examine the roles of

CMA-mediated regulation of MEF2 in these other functional contexts in the nervous system.

In addition to the nervous system, a wider range of other tissues has been shown to express various isoforms of MEF2. For example, in addition to the well documented presence of MEF2s in skeletal muscle, smooth muscle, cardiac muscle, vascular endothelial cell and white blood cells, MEF2s have recently been reported to be expressed in adipose tissue, bone and liver,⁴⁻⁶ just to name a few. Under physiological conditions, MEF2s, as nuclear transcription factor, regulate target gene expression to direct or modulate a host of cellular processes. Regulation of MEF2 levels has been associated with cellular response to a number of pathological stimuli. Evaluating whether CMA-mediated turnover of MEF2 is involved in these other processes is likely to yield valuable insights.

Studies have shown that the stability of MEF2D is also regulated by ubiquitination-proteasome system (UPS), which may play a role in modulating membrane depolarization-induced neuronal survival.⁷ UPS and autophagy pathway are linked and this connection appears to be important for neurons to utilize autophagy capacity to rescue toxicity when UPS is inhibited.⁸ Although CMA is not specifically studied in the aforementioned work, the fact that MEF2D and possibly other MEF2 isoforms are dually regulated by autophagy and UPS makes them ideal candidates to probe the interplay between these two important processes.

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Cell Cycle Features:

RNA polymerase plays both sides

Vivid and bidirectional transcription around and upstream of active promoters

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The transcriptome is a term coined to describe the body of RNA transcripts derived from an organism's genetic material. Recent technical advances have accelerated the cataloguing of transcriptomes of a wide range of species to reveal a common yet puzzling complexity (reviewed in refs. 1 and 2). First the majority of genomic regions previously thought to be transcriptionally inert give rise to a variety of RNAs. Second, regions that were expected to be exclusively transcribed in one orientation overlap additional transcripts that are often produced from the opposite strand of the DNA. While specifics of the function and ultimate fate of most of these newly discovered RNAs remain largely unknown, one underlying pattern is emerging from a set of recent publications: an abundance of short transcripts around, or at a limited distance upstream of, the transcription start sites (TSSs) of known genes.³⁻⁹ In the following, we will discuss the commonalities, differences and implications of these new and surprising findings.

Of the five publications that studied the transcriptomes of different human cell types, four involved high throughput sequencing strategies that allowed the authors to determine the orientation of the transcripts.^{3-5,9} The fifth study used hybridization to tiling microarrays with the added twist of stabilizing RNAs that would otherwise go unnoticed by incapacitating the major eukaryotic 3'-5' RNA degradation machinery; the RNA exosome.⁶ Finally, two publications used a related approach in the yeast *Saccharomyces cerevisiae*.^{7,8}

The studies from the Lis and Sharp laboratories^{3,4} were conducted on different human cell lines and employed very different experimental approaches, yet came to surprisingly similar results (Fig. 1A, top). While the Sharp laboratory subjected short RNAs to massive parallel sequencing, the Lis laboratory devised a method to catch RNA polymerase II (RNAPII) in the act and then analyzed its associated nascent transcripts. The latter approach yielded in addition to abundance and orientation of transcripts also information on the density of active polymerases on the DNA template. In both studies a sharp RNA peak was found an average 50 nt downstream of the TSSs of genes. This

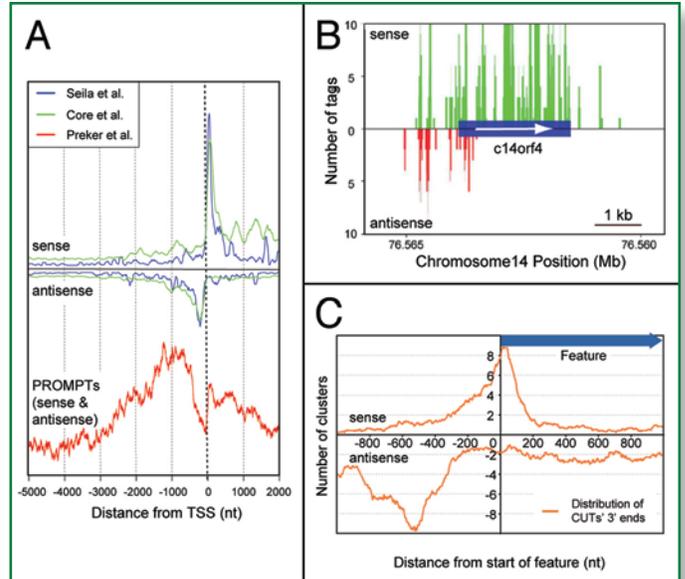


Figure 1. (A) Comparison of the sequence tag distribution from Seila, et al.⁴ (blue curves, www.sciencemag.org/cgi/content/full/1162253/DC1, "human_TSSa-RNA_track"), Core, et al.³ (green curves, Gene Expression Omnibus accession number GSE13518) and microarray data taken from Preker, et al.⁶ (red curve, GSE12431) relative to the TSSs of annotated RefSeq genes (hg17) present in the ENCODE region. Note that the sequencing tags were divided into sense and antisense (above and below the horizontal line, respectively), and that the resulting curves were smoothed over a 50-bp window. (B) Example of tag distribution in the sense (green) and antisense (red) direction for the intronless gene *c14orf4*. Panel taken from ref. 5 with the permission from the American Association for the Advancement of Science. (C) Distribution of RNA 3'-end tag clusters relative to the start of features. Shown is the smoothed distribution of the distances between the start of features (start codons for ORFs) and the nearest CUT clusters. The panel, modified from ref. 7, was kindly provided by Alain Jacquier, Institute Pasteur.

could be explained by earlier findings that the transcription machinery "stalls" at the transition from a slow initiation—to a faster elongation—phase, or might even abort transcription at this position.^{10,11} More surprisingly, however, was the finding that upstream of the TSS an antisense RNA peak trailed away in the opposite direction (Fig. 1A, top). Similar observations had been made two years earlier by the Gingeras laboratory,¹² albeit at a lesser resolution due to the use of microarrays. Now the same group has confirmed and further expanded these results,⁹ again in good agreement with the Lis and Sharp laboratories. Using a cleverly designed method to assay the directionality of transcription on a genome wide scale, the group around Kinzler came to the same conclusion, namely that there is a concentration of antisense tags upstream of many TSSs (Fig. 1B).⁵ For simplicity we will refer to these transcripts collectively as "divergent transcripts".

Finally, the fifth paper investigated stabilization of RNA following siRNA-mediated knock-down of the RNA exosome within ~1% of the human genome by tiling microarrays.⁶ Surprisingly, this revealed a major stabilization of transcripts expressed from a broad region upstream of the majority of active genes, but further away from the TSS than the peaks of antisense RNAs observed in the other studies

(Fig. 1A, bottom red graph). Moreover, manual inspection of several of these so-called PROMoter uPstream Transcripts (PROMPTs) revealed that they generally derive from both strands of the DNA template. Despite their physical separation, PROMPTs share many similarities with the divergent transcripts: (1) they are covered by RNAPII and other markers of transcription initiation;^{4,6,9} in contrast, dimethylation of lysine 79 on H3, an elongation marker, is exclusively present on the gene.⁴ (2) their peak strength correlates well with the activity of the downstream gene,^{3,4,6,9} and both are especially prominent at promoters containing CpG islands.^{3,4,6} (3) their ends are ill defined: while at least a fraction of PROMPTs ends in a poly- or at least an oligo- (A) tail, it has not formally been shown whether PROMPTs and/or divergent transcripts possess canonical 5' cap structures or are generated by processing and then receive a "5' modification analogous to a cap structure" as suggested by ref. 9. (4) they are of low abundance and high heterogeneity under normal conditions, and their mechanism(s) of termination or the sequences that guide them have so far resisted scrutiny (see below).

These observations are not unique to humans: in *S. cerevisiae*, Cryptic Unstable Transcripts (CUTs) are dispersed, short and short-lived transcripts that were at the time of their discovery equally elusive.¹³ In a recent issue of *Nature* two laboratories present a comprehensive catalog of yeast CUTs, again using exosome-deplete conditions (specifically a deletion of the nuclear Rrp6p component) as a tool to stabilize them.^{7,8} By using tiling microarrays and high-throughput sequencing, respectively, the Steinmetz and Jacquier groups show that CUTs are heterogeneous in size but rarely exceed 300 nt, and that they originate bidirectionally from nucleosome free regions (nfrs) found in intergenic spaces, most notably in close vicinity to the ends of genes. In fact, the distribution of sequence tags over the TSS is strikingly similar to that seen in mammals (compare Fig. 1C and A, top).

Are divergent transcripts and PROMPTs related? One possibility is that they are separate entities yet involved in the same function(s). Alternatively, the divergent transcripts could be degradation intermediates or end products of exosome activity acting on longer antisense transcripts. Finally, while divergent transcripts and CUTs all argue for the general bidirectionality of promoters, PROMPTs could be the manifestation of a different property of promoters, possibly that they border "loose" chromatin that readily gives access to RNAPII. Interestingly, loose interactions between histones and DNA may be caused by negative supercoiling created by RNAPII advancing in the sense direction.⁴ Perhaps this would suffice to allow the enzyme to initiate without directional preference and without the need for a bona fide promoter even at distances as far upstream the TSS as the PROMPT region. In contrast, in the case of divergent transcripts and CUTs, TATA-binding protein (TBP) and other general transcription factors might simply be insufficient to direct RNAPII into the "proper" direction.

Another open question is whether degradation of PROMPTs by the exosome is a consequence or the cause of a failure of the transcription machinery to transition into productive elongation. In other words, could the exosome be part of a mechanism that prevents these transcripts to proceed into the "wrong" direction? Such a role would be akin to the quality control mechanism that occurs near the 3' end of faulty gene transcripts.¹⁴ It also remains unknown how divergent transcripts are terminated. The answers might partially come from yeast where a set of factors involved in transcription termination and degradation of CUTs are known. In addition to Rrp6p, these are the Nrd1p/Nab3p/Sen1p and the so-called TRAMP complexes (reviewed in ref. 15). Recently, it was shown that the phosphorylation status of the carboxy-terminal domain (CTD) of the largest subunit of RNAPII is

also important for transcription termination of CUTs.¹⁶ These findings will undoubtedly inspire experiments in human cells.

What, if any, might the biological role of this lavish transcriptional activity be? It might provide a pool of active RNAPII that could be rapidly recruited to the gene proper. Another not mutually exclusive possibility is that transcription upstream of promoters facilitates gene expression by either recruiting chromatin remodeling factors and/or transcription factors through direct interaction or as an indirect result of the act of transcription itself. Aside from these more general roles, PROMPTs might exert specific regulatory function on certain genes. Examples of regulatory RNAs produced in the vicinity of promoters are surfacing in the literature,¹⁷ but no common theme has emerged. In one of the new publications the authors show that expression of Promoter-Associated Small RNAs (PASRs) in trans can lead to mild downregulation of *c-MYC* gene expression,⁹ while we found that an increase in the steady-state level of PROMPTs correlates with an increase in the degree of CpG methylation in the cognate promoter.⁶ Thus like many intronic sequences some PROMPTs might have been co-opted in different ways to increase genetic flexibility. An extreme of this idea would be that sense PROMPTs might eventually give rise to new 5' exons as suggested by occasional ESTs that span from the PROMPT region into the downstream gene. Indeed, every new such discovery challenges the early geno-centric view anew.

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Cell Cycle Features:

G₁ to S transition and pluripotency

Two sides of the same coin?

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'I reasoned that study of the cell cycle responsible for the reproduction of cells was important and might even be illuminating about the nature of life,'—Sir Paul Nurse, biochemist and identifier of Cdc2 control of G₁ to S phase progression in yeast.

The cell cycle is a collective term for the series of events taking place in a cell that culminates in its replication. The process comprises four distinct phases (G₁, S, G₂ and M phase) all of which are tightly regulated through the action of a large number of proteins including Cyclins and Cyclin Dependent kinases. Strictly speaking there is a fifth phase called G₀ but since this is only populated by cells that have temporarily stopped dividing it is often neglected in discussions of cell cycle control mechanisms (reviewed in ref. 1). G₁ phase (sometimes called Gap1) has fascinated biologists for a long time for it is during this stage that synthesis of various enzymes that are mostly required for DNA replication in S phase occurs and specification of replication origins sites that enable replication in S phase is determined.² If problems arise in any of these systems then molecular checkpoints will prevent the cell progressing to S-phase however it is also during this stage that the cell makes key decision to self-renew, differentiate or die based on complex signaling obtained by its microenvironment. The duration of G₁ is variable, even amongst different cells of the same species. G₁ is particularly short in embryonic stem cells (obtained from preimplantation embryos) of different species including mouse, primates and human.³⁻⁵ The biological significance of these observations is not fully understood yet, but several key questions emerge from these studies and some of those are outlined below.

What is the biological significance of short G₁ phase and does this play a role in maintenance of pluripotency? We know that neural progenitor cells can be divided into two subpopulations based on the length of their cell cycle. Those that are simply undergoing mitotic proliferation have a shorter cycle than those committed to undergo neuronal differentiation⁶ but the really

interesting finding from these studies is that similar differentiation of neural progenitors to neuroepithelial cells can be induced by lengthening of G₁ phase alone.⁷ Is the short G₁ phase a pre-requisite for stem cell self renewal? Data from pluripotent embryonic stem cells would seem to support this possibility as our own studies of human embryonic stem cells have shown that downregulation of a single Cyclin Dependent kinase (CDK2) can cause a drastic change in cell cycle regulation of these cells resulting in accumulation of cells in G₁ phase. The biological impacts of this are significant: cells lose the typical morphology of embryonic stem cells and begin to express differentiation specific genes.⁸ Even if the downregulation of CDK2 was only transient its effect upon the cell is permanent for even though the cells are able to re-enter the cell cycle as the levels of CDK2 rise back to those normal for embryonic stem cells, the genes associated with the pluripotent phenotype never return to their former levels. Moreover, the persistent expression of differentiation associated genes confirms that reacquisition of a short G₁ phase cannot reverse the differentiation marks established during the lengthening of G₁ phase.⁸ Similar G₁ lengthening occurs during spontaneous embryonic stem cell differentiation and suggests that a longer G₁ is perhaps necessary for establishment of epigenetic marks (removal of H3 and H4 acetylation and establishment of repressive H3K9 dimethylation and trimethylation, ref. 9) that are necessary for initiation of the differentiation process. An essential feature of the epigenetic modifications that accompany differentiation is that they are largely irreversible therefore any gene expression profiles controlled in this manner will be unlikely to change when G₁ shortens unless this change can somehow reverse epigenetic modification of the genome.

What are the molecular mechanisms that govern such fast G₁ to S transition? Since a short G₁ is linked to maintenance of pluripotency, it is reasonable to speculate that the core transcriptional machinery that underlines the pluripotent phenotype may also be responsible for a fast G₁ to S transition in stem cells. It is now well accepted that three key pluripotency factors, OCT4, SOX2 and NANOG which are essential for propagation of undifferentiated embryonic stem cells work in concert to activate or repress a substantial portion of their target genes which are essential to maintenance of pluripotency or differentiated state.¹⁰ It is perhaps not surprising therefore the list of target genes activated by one or more of these transcription factors includes key players of cell cycle regulation such as CDK1, CYCLIN D1, CDK4, CDC7,¹⁰ suggesting direct involvement of pluripotency factors in the regulation of their transcription. Despite the observation that downregulation of OCT4, SOX2 and NANOG in human embryonic stem and carcinoma cells causes expression changes in a significant number of genes involved in cell cycle regulation, direct interaction has only recently been confirmed by our group¹¹ when we showed that NANOG binds directly to the promoter region of CDC25A (a phosphatase involved in both G₁ and G₂ regulation) and intragenic regions of CDK6 (Cyclin Dependent kinase important for G₁ to S transition), resulting in their transactivation. We further confirmed that these downstream transcriptional effectors are directly responsible for modulating the length of G₁, thus establishing for the first time a direct link between length of G₁, in embryonic stem cells and regulators of pluripotency. This is however only the beginning of our understanding since a new class of regulators, microRNAs (miRNAs) have also been shown to regulate the length of G₁ phase in embryonic stem cells.¹² One of the most prominent miRNA clusters is miR-290 which is expressed in murine embryonic stem cells and shown to modulate G₁ to S transition by repressing one of the key inhibitors, p21.¹³ Similarly, the miR-302-367 cluster which is highly expressed

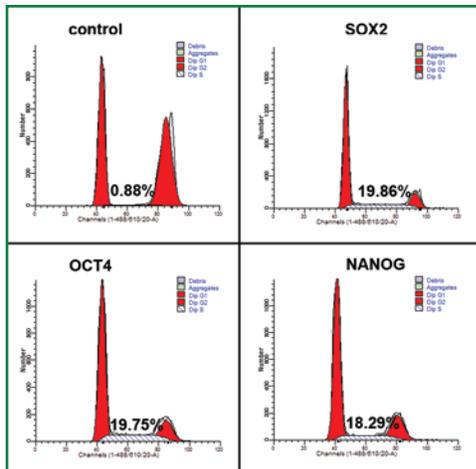


Figure 1. Increase in fraction of cells in S phase 48 hours after retroviral mediated overexpression of *OCT4*, *SOX2* or *NANOG* in neonatal human dermal skin fibroblasts. Propidium iodide staining followed by flow cytometry analysis (BD LSR II) is shown.

in human embryonic stem cells and whose expression is regulated by *OCT4*, *SOX2* and *NANOG*¹⁴ has been shown to regulate G₁ to S transition through post translational regulation of Cyclin D2 expression.¹⁵ It must be said that the changes caused in cell cycle regulation by the action of miRNAs are modest compared to impacts of transcription factors such as *NANOG* suggesting that miRNAs are more likely to act as tuners of cell cycle.

What are the implications of G₁ to S transition for the induction of pluripotency? 'A discovery is said to be an accident meeting a prepared mind.'—Albert Szent Gyorgyi (1893–1986).

Revolutionary studies in the stem cell field have shown that it is possible to reprogram pluripotency in somatic cells by overexpression of key pluripotency factors such as *OCT4*, *NANOG*, *SOX2*, *LIN28*, *KLF4* and *c-MYC*. During the reprogramming process, one of key change that must occur is the shortening of G₁ phase to a length more appropriate to pluripotent cells. Our own preliminary data (Fig. 1) suggest that changes in cell cycle are likely to happen very early during the reprogramming process even before the cells start to re-express their own pluripotency markers. Although a direct transactivation of cell cycle regulators by key pluripotency factors in somatic cells has not been proven as yet, the short time required to increase the fraction of cells in S phase during the transduction of these factors suggest that this is likely to be a direct effect rather than a facet of some more general reprogramming mechanism. It is therefore likely that interference with cell cycle inhibitors or key tumor suppressors that govern the G₁ to S transition may be able enhance the reprogramming ability of this currently rather inefficient process. This possibility is supported by the enhancement of induced pluripotent cells following the inhibition of the regulator p53 in the absence of *C-MYC* expression¹⁶ and if such alternative mechanisms can be found to generate induced pluripotent stem cells that do not rely upon the current use of retroviral vectors, clinical application of this reprogramming effect will become a realistic possibility. Given the enormous benefits to be had from this work it would be surprising if the field did not develop rapidly but we believe that a greater understanding of the cell cycle dynamics of pluripotent cells can contribute in many ways towards the development of technologies to improve human health.

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Cell Cycle Features:

Sgt1 plays polo during cell division

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Sgt1 was firstly described as a supressor of Skp1, a component of the SCF ubiquitin ligase complex, and is characterized by having three different domains, the tetratricopeptide repeat domain (TPR), the CHORD and the Sgt1-specific central domain (CS) and the Sgt1-specific domain (SGS). Both TPR and CS Domains are important for the binding of Sgt1 to the chaperone Hsp90 and in this context plays an essential role for kinetochore assembly both in yeast and human cells,¹ plant disease resistance pathway² and in inflammatory responses in higher eukaryotes.³ The importance of Sgt1 in cell cycle progression was first highlighted when yeast cells carrying a Sgt1 temperature sensitive allele were shown to delay G₁/S and G₂/M transition.

Analysis of yeast mitotic cells revealed that the G₂/M transition phenotype was caused by impairment of the kinetochore assembly pathway demonstrating an important role in cell division.¹ Currently, it is thought that dimers of Sgt1,⁴ bind to the chaperone Hsp90 and that this complex increases the ability of Sgt1 to associate and activate Skp1, directing it to Ctf13 an essential kinetochore component. Subsequently, Ctf13 binds other components of the CBF3 complex which forms the core of the yeast kinetochore and is the first step in the assembly of kinetochores in *S. cerevisiae*.⁵ Sgt1 has also been shown to be important for kinetochore assembly in Human cells,⁶ as depletion of Sgt1 by RNA interference in HeLa cells results in abnormal kinetochore structure and a number of outer kinetochore proteins such as Hec1, CENP-E, CENP-F and CENP-I fail to accumulate resulting in abnormal microtubule kinetochore attachment and failure of chromosome congression.⁶ Interestingly, kinetochore accumulation of proteins involved in the spindle assembly checkpoint (SAC), such as BubR1, Mad1 and Mad2, is also impaired and although cells are delayed in prometaphase, the SAC is significantly weakened.⁶

Similar effects upon kinetochore assembly were also demonstrated after either depletion or inhibition of Hsp90, phenotypes that were suppressed by overexpression of Sgt1.⁷ Taken together these results suggest that the Sgt1-Hsp90 complex has a conserved role in the kinetochore assembly pathway in these species. To determine if a similar pathway also operates in *Drosophila* we analyzed cell cycle progression and cell division in third larval neuroblasts isolated from flies that carry a mutant allele of the only gene that encodes the SGS-domain

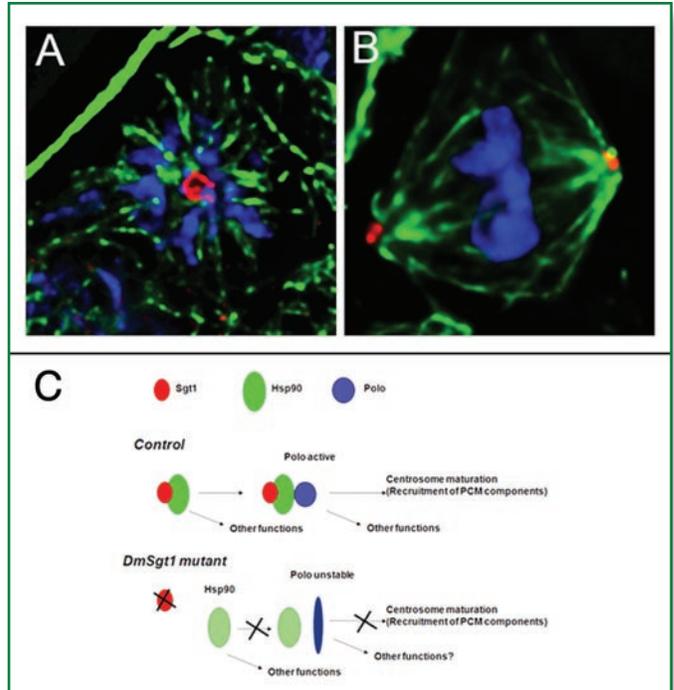


Figure 1. Organization of the mitotic apparatus in Sgt1 mutant *Drosophila* neuroblasts before and after overexpression of Polo; Third instar larval *sgt1^{P1}* mutant neuroblasts were isolated before (A) or after (B) overexpression of Polo, fixed and immunostained to reveal DNA (Blue), tubulin (Green) and centrosomes (Red); (A) Typical Sgt1 mutant cell showing a monopolar spindle with a single centrosome at the center of the aster; (B) Sgt1 mutant cell after overexpression of Polo kinase rescues the mitotic phenotypes and show spindles that are bipolar with two well-defined centrosomes at the poles; (C) General model describing the role of the Sgt1-Hsp90 complex in Polo stability/activity.

in this organism.⁸ Unlike yeast and humans, *Drosophila* Sgt1 contains the SGS and CS domain but does not contain the TPR domain known to be required for its dimerization.⁴ Although there is a consensus that Sgt1 and Hsp90 interact, different studies have shown that this interaction occurs through different domains in different species so that in yeast, Sgt1 interacts with Hsp90 mostly by the TPR Domain^{9,10} while in metazoans it uses the CS Domain.¹¹ Sgt1 is an essential protein in *Drosophila* since homozygotes individuals are not viable and die during late larval stages that are characterized by extensive diploid cell proliferation. Surprisingly, analysis of kinetochore organization in mutant neuroblasts showed that unlike yeast and human cells, in *Drosophila*, kinetochore organization appeared mostly normal in mutant cells, including the localization of proteins like CENP-E and CENP-C and also SAC proteins BubR1, Bub1 and Bub3.

Moreover, mutant cells show significant mitotic delays during prometaphases as shown by *in vivo* studies and by severe chromosome hypercondensation, suggesting an active SAC response. However, while analyzing other kinetochore proteins we observed that the levels of Polo kinase were significantly reduced both at kinetochores and in total cell extracts. Polo kinase was first studied in detail in *Drosophila*¹² and has subsequently emerged as a key mitotic regulator. Importantly, it has been shown that the stability of Polo is directly linked to Hsp90 activity.¹³ While the role of Polo at kinetochores is

still under debate, it is known to be essential for centrosome maturation and spindle organization for which its interaction with Hsp90 appears to be essential.¹³ Thus, we turned to analyze the organization and function of the mitotic apparatus in Sgt1 mutant cells. Our results demonstrate that in the absence of Sgt1, centrosome maturation is mostly impaired and spindle organization is aberrant since a significant proportion of cells form monopolar spindles, phenotypes that are highly reminiscent of either mutations in Polo or inhibition of Hsp90.

A link between Polo and Sgt1 was clearly demonstrated when we overexpressed wild type Polo in a sgt1 mutant fly (Fig. 1). Using a weak larval promoter we observed that centrosome maturation, spindle bipolarity and mitotic progression were significantly rescued. These results demonstrate that in *Drosophila*, Polo is a major target of the Sgt1-Hsp90 chaperone complex.⁸ Further studies are now needed to clarify why *Drosophila* Sgt1 is apparently not required for kinetochore assembly.

Drosophila Sgt1 contains a CS domain that should allowed it to interact with Hsp90 but does not contain the TPR domain essential for binding Skp1 and subsequently for kinetochore assembly in other species. Additional TPR-containing proteins might be required for this process in flies.

However, it is interesting that the mitotic phenotypes resulting from mutation or depletion of Sgt1 in yeast, human and *Drosophila* share common features, including delays during mitotic progression, abnormal spindle function and failure of chromosome congression suggesting that the SAC is at least partially active. Thus, it would be essential to determine whether Sgt1 is also required for the stability and localization of Polo homologues in yeast and human cells, given that inhibition of Hsp90 decreases the activity of Plk1 in HeLa and in some tumor cells.¹⁴

Accordingly, whether Sgt1 plays Polo only in flies or also does it in other species remains to be determined.

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