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Function of the INO80 chromatin remodeling complex in DNA replication

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1. ABSTRACT

ATP-dependent chromatin remodeling complexes are involved in several nuclear processes. In particular the INO80 remodeling complex is an essential factor during transcription and DNA repair. Recently, several labs have described a novel role for INO80 during DNA replication. Moreover, Falbo *et al*. have presented evidence linking INO80's activities to the DNA damage tolerance pathways during replication (1). In this review we will discuss and integrate the results obtain by these various research groups to describe a novel role for INO80 in DNA replication.

2. INTRODUCTION

DNA replication, an essential and complex process required to produce an accurate copy of the genetic material, has been widely investigated in the past decades. In higher eukaryotes the DNA is not naked, but wrapped around histone proteins in a highly compacted structure called chromatin. During replication, the chromatin structure needs to be relaxed to an "open" state so that proteins and factors actively involved in this process can access the DNA molecule. Moreover, the correct and precise coordination between DNA replication and chromatin structure regulation is indispensable to ensure

that DNA replication occurs without errors that could eventually lead to diseases such as cancer.

In the eukaryotic nucleus the DNA molecule is wrapped around histones to form a basic unit, the nucleosome. Nucleosomes interact with each other creating a highly compacted and complex structure that creates impediments and constraints to any process that requires access to the DNA molecule, such as DNA replication (2). In the past decade, several lines of evidence have indirectly suggested that regulation of the chromatin structure is a key process during DNA replication (3). Nucleosome positioning has been shown to be important during replication initiation and previous research indicates that regulation of the chromatin structure is necessary for correct chromatin maturation at the replication fork (4) (5) (6). However, until recently, very little was known about how the chromatin environment affects and regulates DNA replication.

The correct inheritance of the DNA sequence requires a highly regulated but dynamic environment that ensures rapid yet selective access to the DNA molecule (3). A diverse array of chromatin modifying factors work together to achieve this tightly regulated environment where nucleosomes can be mobilized along the DNA molecule, exchanged, evicted, or posttranslationally modified to allow accurate completion of the many nuclear processes that involve the chromatin. Among these factors, chromatin remodeling complexes use the energy provided by ATP to mobilize, exchange or evict nucleosomes (7). In particular, a subfamily of chromatin remodeling complexes, the INO80 subfamily, has been shown to be an important player in nucleosome mobilization during transcription, although the pertinent mechanism is still not clear (8). The INO80 complex binds to the promoter regions of certain actively transcribed genes and mobilizes nucleosomes using the energy provided by ATP. Remarkably, some recent observations suggest that the INO80 chromatin remodeling complex might be directly involved in DNA replication. For instance, *ino80* mutants are sensitive to hydroxyl-urea (HU) and methyl methanesulfonate (MMS), two well characterized DNA replication blocking agents, suggesting INO80 could be directly involved in DNA replication, particularly through regulation of the chromatin structure, to avoid replication related DNA damage (9) (10). Based on this observation several papers have been published in the past two years addressing a novel role for the INO80 remodeling complex in DNA replication (1) (11) (12) (13). The focus of this review is to summarize these new findings that reveal novel functions of the INO80 remodeling complex in DNA replication.

3. A NOVEL ROLE FOR INO80 IN DNA REPLICATION

3.1. INO80 is involved in DNA replication

The HU sensitivity of the *ino80* mutants recently led several labs to investigate, using different experimental approaches, whether INO80 affects DNA replication directly, since the marked increase in Ino80 expression observed in cells that were synchronized in G1 and then released into the S phase in media with HU strongly suggested that INO80's role in DNA replication could be direct instead of transcription-related (1) (11) (12) (13). In fact, strong evidence in support of a direct role for INO80 during DNA replication arose recently from four different research groups that analyzed INO80 binding to origins of replication (ARS) in yeast. Using Chip-ChIP analysis to investigate INO80's binding profile on *S. cerevisiae* chromosomes 3, 4, 5 and 6, Shimada *et al.* described that INO80 binds preferentially to active early ARSs in cells released into the S phase in media with HU (12). In addition, a whole genome Chip-ChIP analysis performed in S phase synchronized cells by Falbo *et al.* indicated that INO80 binds to 45% of known ARSs and that this binding is distributed along all yeast chromosomes, suggesting INO80 is an important factor during replication genome-wide. Interestingly, INO80 seems to be broadly required for both early and late firing origins, since it binds to 55% of early ARSs and 45% of late ARSs genome-wide (1). Despite the differences with Shimada's data, these results are supported by data obtained by Vincent el al. showing that INO80 is necessary for the replication of late firing origins. Moreover, INO80's binding to ARSs was shown to be S phase specific. INO80 binds to only 4% of total ARSs in G2 nocodazole-arrested cells, where INO80 is expressed at levels similar to those in S phase HU-arrested cells. Interestingly, analysis of microarray data originated from *ino80* mutant cells synchronized in S phase and treated with MMS indicated that only 6.5% of the INO80 binding signal at ARSs correlates with promoter regions of genes known to be transcriptionally regulated by INO80, supporting the idea that INO80's presence at ARSs is not related to transcription. These results are in agreement with a model in which INO80 binds genome-wide to ARS, probably to directly perform a replication-related function.

3.2. INO80 and the checkpoint response

The hypersensitivity of the *ino80* mutant to DNA replication-blocking agents, such as HU, strongly suggested that INO80 could be involved in the response to stalled replication forks (10). In yeast, HU induces replication fork stalling in cells released from G1 arrest into the S phase (14). In the presence of HU, the Rad53-dependent intra-S phase checkpoint is activated by Rad53 phosphorylation that leads to fork stabilization and inhibition of late firing origins. Interestingly, despite the well-established sensitivity of the *ino80* mutants to HU, data originated in several labs have generated some diverse conclusions on whether or not INO80 is involved in replication fork stability and the S phase checkpoint response.

The most direct way to show a defect in replication fork stabilization after HU treatment is 2D gel analysis, since it is well established that in mutants of proteins involved in replication fork stabilization, such as Rad53, replication forks collapse resulting in the accumulation of DNA fragments that form a characteristic cone signal when assessed by 2D gel electrophoresis. Interestingly, 2D gel analysis of *ino80* mutants after HU treatment has shown dissimilar results. In a recent report published by Falbo *et al.*, the authors tested whether *ino80* mutants exhibited replication fork defects similar to those

typical of *rad53* mutants by 2D gel analysis and found that, unlike the *rad53* mutant, both Wild Type and *ino80* mutants were proficient in replication fork maintenance and stabilization, as well as in the repression of late firing origins (1). Interestingly, in a different report by Papamichos-Cronakis *et al*., it was concluded that the absence of Ino80 leads to replication fork collapse, based on an experiment where the bubble signal is not clearly present, and a positive control, such as *rad53*, is absent; thus the conclusion somewhat uncertain (11). However, since a single ARS is not representative of a whole genome, Falbo *et al.* examined the phosphorylation status of histone H2AX, a well-established marker of DSB formation that could arise globally from other collapsed replication forks. The authors found no significant differences in the phosphorylation pattern of Wild Type and *ino80* synchronized cells released from HU arrest, suggesting that the lack of replication fork collapse after HU treatment is global rather than a single isolated event (1). Finally, Shimada *et al.* described that *ino80* mutants have a slight delay in the recovery from replication fork arrest when cells are released from HU arrest. This result is in concordance with the fact that *ino80* cells also seem to present a few repair foci enriched in Ddc2-Mec1, indicative of DNA repair activity (12). However, although it is possible that a very small cone signal could have gone undetected in the 2D gel analysis, a delay in replication fork completion, instead of a cone signal, could not be representative of replication fork collapse. Nonetheless, it is possible that INO80 plays a role in the stabilization of only a subset of ARSs without affecting the global response to HU. These results, although somewhat different in the details, are not entirely surprising, since INO80 has been shown to bind to both early and late firing origins, while accumulation of a cone signal resulting from collapsed forks was described as an attribute limited only to early ARS. Furthermore, this idea is supported by the data presented in a report by Vincent *et al.* showing that both Isw2 and Nhp10 seem to be necessary for proper replication of late firing origins (13). Since Nhp10 is an INO80 exclusive subunit, these data support the notion that INO80 could have the same or even different functions at both early and late firing origins.

3.3. A novel role for INO80 in the DNA damage tolerance pathway

The MMS sensitivity of the *ino80* mutants led Falbo *el al.* to hypothesize that INO80 could be necessary to avoid replication related DNA damage mediated by the Rad18/Rad6 DNA damage tolerance pathways (1). In yeast, replication forks that encounter a DNA obstruction, such as an MMS induced adduct, stall. When the MMS induced damage is in the template for the leading strand, a gap will be formed that cannot be filled by the polymerase, and subsequent excision of this region creates a DSB by destruction of the replication fork (15). Bypass of these lesions is mediated by activation of the *RAD18/RAD6* DNA damage tolerance pathway, and mutants of proteins involved in this pathway are, similar to the *ino80* mutant, sensitive to MMS (16). In addition, the authors found that *ino80* mutant cells synchronized in G1 and treated with MMS accumulate the phosphorylated form of histone H2AX (γH2AX), a marker of DSBs, only when the cultures are allowed to progress through the S phase, indicating that INO80 is necessary to avoid DNA damage generation during replication (1).

Since INO80 was shown to bind ARSs directly, the authors examined whether the γH2AX accumulation observed in the *ino80* mutant was directly related to deficient replication fork activities. Thus, to provide direct conclusive evidence, a state-of-the-art technique, DNA combing, was used to examine replicating DNA at the single molecule level; thus overcoming the pitfalls of other techniques. Analysis of Wild Type and *ino80* mutant cultures synchronized in S phase and released from MMS treatment indicated that the length of BrdU tracks, indicative of replication fork movement, was significantly reduced in the *arp8* mutant (Arp8 is an INO80 subunit whose deletion closely resembles the Ino80 subunit deletion). Moreover, these data were supported by PFGE analysis of the same samples, showing a marked reduction in chromosome mobility in agarose, and the persistence of un-replicated gaps observed by DNA combing. Therefore, Falbo el al. established that INO80 is required for efficient replication fork reestablishment and progression after MMS treatment (1).

3.4. INO80 is necessary for PCNA ubiquitination and Rad18 recruitment to replication forks

Interestingly, the observation that the *rev3rad30* double mutant, a strain that lacks the major translesion polymerases, shows the same defect as the *ino80* mutant after release from MMS assessed by combing, led Falbo *et al*. to hypothesize that such defect could be a consequence of a deficient PCNA ubiquitination, since translesion polymerases depend on PCNA ubiquitination for activation (16). In yeast, the primary system activated to overcome MMS induced lesions requires proper PCNA ubiquitination and is mediated by proteins that belong to the *RAD6/RAD18* DNA damage tolerance pathways. Mutants of proteins involved in these pathways are sensitive to MMS and have difficulty resuming DNA replication after MMS treatment, similar to the *ino80* and *arp8* mutants. In budding yeast, these pathways are dependent upon Lys 164 ubiquitination of PCNA (K164). Monoubiquitination of K164 by Rad6-Rad18 leads to the activation of an error free damage avoidance pathway, while the subsequent polyubiquitination of K164 by the Mms2-Ubc13-Rad5 enzyme complex leads to the activation of an error free damage avoidance pathway (16). Interestingly, a point mutant with a substitution in K164 that is deficient in PCNA ubiquitination (K164R) is also sensitive to MMS. Thus, to investigate whether PCNA ubiquitination is affected by INO80, the authors assessed the PCNA ubiquitination status in S phase synchronized, MMS treated *ino80* mutant cultures. Immunoprecipitation with a PCNA antibody from trichloroacetic acid extracted proteins, followed by a Western blot using an anti-ubiquitin antibody showed that, unlike the unmodified form of PCNA, the ubiquitinated forms were consistently reduced in the *ino80* mutant as well as in a point mutant that specifically lacks the ATPase activity, leading to the conclusion that INO80

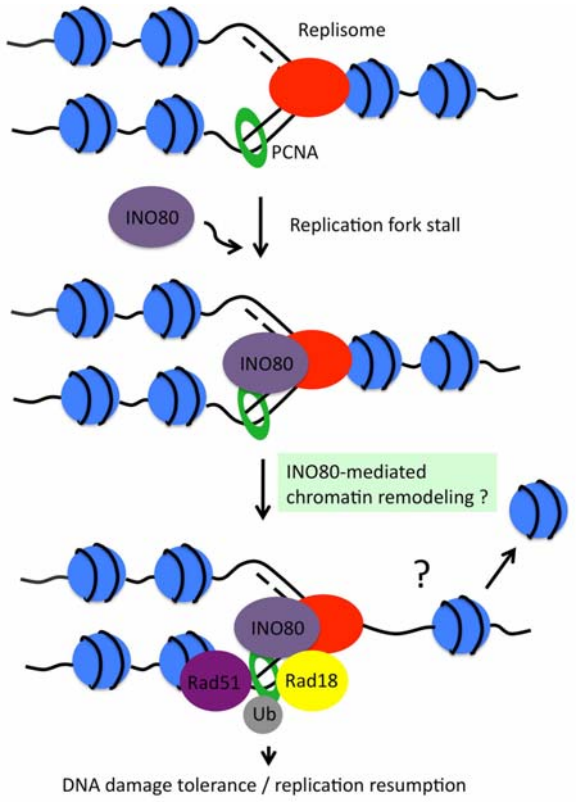

Figure 1. The role of INO80 in DNA damage tolerance during replication.After replication fork encounters DNA replication insults, the INO80 chromatin remodeling complex is recruited to the blocked replication fork. INO80 chromatin remodeling activity remodels the local chromatin environment to facilitate the recruitment of DNA damage tolerance factors, such as Rad18 and Rad51. These initiating factors activate subsequent pathways to resolve the DNA damage-induced blockage at the replication fork, allowing proper DNA replication resumption.

is necessary for PCNA ubiquitination, in a process that possibly requires INO80 ATPase and remodeling activities (1).

INO80 could be involved in PCNA ubiquitination by affecting proper recruitment of proteins that mediate the damage tolerance pathways, such as Rad18 or Rad6. Interestingly, a whole genome expression profile analysis using microarrays showed no significant differences in the expression of genes known to be involved in the damage tolerance pathways, suggesting the effect could be direct, instead of transcriptionally related. Indeed, the authors investigated Rad18 recruitment to defined ARSs after MMS treatment and found that while recruitment of a bona fide replication factor such as Rfc3 was similar in Wild Type and *ino80* cultures, Rad18 recruitment was significantly reduced in the *ino80* mutant strain during S phase, indicating that INO80 is necessary for proper Rad18 recruitment and PCNA ubiquitination (1).

3.5. INO80 is necessary for Rad51 recruitment to replication forks and its recombination mediated activities

Remarkably, recent investigations have provided strong evidence indicating that Rad18 might work together with Rad51 in a joint effort to resolve stalled replication forks after MMS treatment. In fact, after replication forks stall, Rad51 is recruited to mediate a recombination process that results in the generation of the so-called hemicatenate-like structures or X-shaped structures, that are subsequently resolved by the activity of a REC helicase, Sgs1. These structures can be visualized using 2D gel electrophoresis, where lack of Rad51 leads to a reduction, while lack of Sgs1 leads to an accumulation of the X-shaped structures' signal. Interestingly, formation of these X-shaped structures was recently shown to be dependent upon PCNA ubiquitination and Rad18 activity (17). Moreover, in support of INO80's role in Rad18 recruitment and replication fork resolution, Falbo *et al.* found that Rad51 mediated X-shaped structures' formation was significantly reduced in cells that lack INO80 activity, leading to the notion that both Rad18 and Rad51 activities are affected by INO80 at replication forks (1). In support of these results, ChIP analysis to assess Rad51 binding to ARSs indicated that Rad51 recruitment to replication forks is reduced in the *ino80* mutant. As such, INO80 could remodel the chromatin at or around the replication fork to allow Rad51 and Rad18 proper access to the DNA in order to process stalled replication forks (1)

4. CONCLUSION

These studies reveal a new and distinct role for INO80 in DNA replication. Although some data seem to indicate that INO80 could be involved in the HU mediated checkpoint activation, further research is required to support this notion. INO80 may have some role in stabilizing specific ARSs under HU stress. Furthermore, data provided in Falbo's study strongly supports a role for INO80 in the DNA damage tolerance pathways after MMS treatment. In fact, these investigations provide strong evidence for a model in which, after replication forks encounter an obstruction, INO80 is recruited to mediate proper Rad18 and Rad51 recruitment that would lead to PCNA ubiquitination and Rad51 mediated fork resolution through a process that involves recombination and possibly template switching. Indeed, these investigations have established INO80 as an early player in modulating multiple pathways that lead to the resolution of replication forks, thus establishing chromatin remodeling factors as a novel event that is required to avoid DNA damage as an undesired consequence of DNA replication (Figure 1).

5. FUTURE PERSPECTIVES

The role of chromatin remodeling during DNA replication has long been suspected, and several recent papers have clearly established that, indeed, chromatin remodeling complexes are required during DNA replication. As such, future research efforts would be directed to understand the many aspects left unresolved in

these studies. It is still unclear how INO80 is recruited to DNA replication sites and what are the signals, factors, and pathways leading to this event. One way INO80 could be directed to ARSs is through the physical interaction with replication factors. Moreover, a very interesting aspect left unresolved is whether INO80 remodels the chromatin near or at the replication forks and whether these remodeling activities regulate Rad18 and Rad51 access to the forks during DNA replication.

Most importantly, INO80 and the DNA damage tolerance pathways are well conserved from yeast to humans (18). Thus, the recent discovery of an homolog of INO80 in humans (hINO80) has opened an exciting new area that could lead to important discoveries, specifically in cancer research, since the damage avoidance responses during DNA replication are essential to preserve genome stability (19). As such, deletion of the human homolog of INO80 (hINO80) was shown to increase the sensitivity of these mutants to DNA damaging agents, and YY1, a transcription factor associated with hINO80, was shown to bind recombination intermediate structures (20). Moreover, a very recent paper by Hur *et al.* provides evidence implicating hINO80 in DNA replication and chromosome segregation, setting the stage for the expansion of a very important novel research area (21). Given the multiple roles of INO80 in the DNA damage response, these studies, together, reveal novel and specific roles of INO80 in diverse aspects of DNA replication.

6. REFERENCE

1. K. B. Falbo, C. Alabert, Y. Katou, S. Wu, J. Han, T. Wehr, J. Xiao, X. He, Z. Zhang, Y. Shi, K. Shirahige, P. Pasero and X. Shen: Involvement of a chromatin remodeling complex in damage tolerance during DNA replication. *Nat Struct Mol Biol*, 16(11), 1167-72 (2009)

2. G. Vernet, M. Sala-Rovira, M. Maeder, F. Jacques and M. Herzog: Basic nuclear proteins of the histone-less eukaryote Crypthecodinium cohnii (Pyrrhophyta): two-dimensional electrophoresis and DNA-binding properties. *Biochim Biophys Acta*, 1048(2-3), 281-9 (1990)

3. K. B. Falbo and X. Shen: Chromatin remodeling in DNA replication. *J Cell Biochem*, 97(4), 684-9 (2006)

4. S. P. Bell and B. Stillman: ATP-dependent recognition of eukaryotic origins of DNA replication by a multiprotein complex. *Nature*, 357(6374), 128-34 (1992)

5. Y. Okuno, H. Satoh, M. Sekiguchi and H. Masukata: Clustered adenine/thymine stretches are essential for function of a fission yeast replication origin. *Mol Cell Biol*, 19(10), 6699-709 (1999)

6. R. A. Poot, L. Bozhenok, D. L. van den Berg, S. Steffensen, F. Ferreira, M. Grimaldi, N. Gilbert, J. Ferreira and P. D. Varga-Weisz: The Williams syndrome transcription factor interacts with PCNA to target chromatin remodelling by ISWI to replication foci. *Nat Cell Biol*, 6(12), 1236-44 (2004)

7. Y. Bao and X. Shen: SnapShot: Chromatin remodeling: INO80 and SWR1. *Cell*, 144(1), 158-158 e2 (2011)

8. Y. Bao and X. Shen: INO80 subfamily of chromatin remodeling complexes. *Mutat Res*, 618(1-2), 18-29 (2007)

9. A. J. Morrison, J. Highland, N. J. Krogan, A. Arbel-Eden, J. F. Greenblatt, J. E. Haber and X. Shen: INO80 and gamma-H2AX interaction links ATP-dependent chromatin remodeling to DNA damage repair. *Cell*, 119(6), 767-75 (2004)

10. X. Shen, G. Mizuguchi, A. Hamiche and C. Wu: A chromatin remodelling complex involved in transcription and DNA processing. *Nature*, 406(6795), 541-4 (2000)

11. M. Papamichos-Chronakis and C. L. Peterson: The Ino80 chromatin-remodeling enzyme regulates replisome function and stability. *Nat Struct Mol Biol*, 15(4), 338-45 (2008)

12. K. Shimada, Y. Oma, T. Schleker, K. Kugou, K. Ohta, M. Harata and S. M. Gasser: Ino80 chromatin remodeling complex promotes recovery of stalled replication forks. *Curr Biol*, 18(8), 566-75 (2008)

13. J. A. Vincent, T. J. Kwong and T. Tsukiyama: ATP-dependent chromatin remodeling shapes the DNA replication landscape. *Nat Struct Mol Biol*, 15(5), 477-84 (2008)

14. M. Lopes, C. Cotta-Ramusino, A. Pellicioli, G. Liberi, P. Plevani, M. Muzi-Falconi, C. S. Newlon and M. Foiani: The DNA replication checkpoint response stabilizes stalled replication forks. *Nature*, 412(6846), 557-61 (2001)

15. F. Z. Watts: Sumoylation of PCNA: Wrestling with recombination at stalled replication forks. *DNA Repair (Amst)*, 5(3), 399-403 (2006)

16. C. Hoege, B. Pfander, G. L. Moldovan, G. Pyrowolakis and S. Jentsch: RAD6-dependent DNA repair is linked to modification of PCNA by ubiquitin and SUMO. *Nature*, 419(6903), 135-41 (2002)

17. D. Branzei, F. Vanoli and M. Foiani: SUMOylation regulates Rad18-mediated template switch. *Nature*, 456(7224), 915-20 (2008)

18. H. D. Ulrich: Conservation of DNA damage tolerance pathways from yeast to humans. *Biochem Soc Trans*, 35(Pt 5), 1334-7 (2007)

19. S. Bergink and S. Jentsch: Principles of ubiquitin and SUMO modifications in DNA repair. *Nature*, 458(7237), 461-7 (2009)

20. S. Wu, Y. Shi, P. Mulligan, F. Gay, J. Landry, H. Liu, J. Lu, H. H. Qi, W. Wang, J. A. Nickoloff and C. Wu: A YY1-INO80 complex regulates genomic stability through homologous recombination-based repair. *Nat Struct Mol Biol*, 14(12), 1165-72 (2007)

21. S. K. Hur, E. J. Park, J. E. Han, Y. A. Kim, J. D. Kim, D. Kang and J. Kwon: Roles of human INO80 chromatin remodeling enzyme in DNA replication and chromosome segregation suppress genome instability. *Cell Mol Life Sci*, 67(13), 2283-96 (2010)

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