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## Structure, function and regulation of Jade Family PHD Finger 1 (JADE1)

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### Abstract

The family of JADE proteins includes three paralogues encoded by individual genes and designated PHF17 (JADE1), PHF16 (JADE2), and PHF15 (JADE3). All three JADE proteins bear in tandem two Plant Homeo-domains (PHD) which are zinc finger domains. This review focuses on one member of the JADE family, JADE1. Studies addressing the biochemical, cellular and biological role of JADE1 are discussed. Recent discoveries of JADE1 function in the regulation of the epithelial cell cycle with potential relevance to disease are presented. Unresolved questions and future directions are formulated.

### I. Introduction

JADE family PHD zinc finger 1 (Gene for Apoptosis and Differentiation in Epithelia 1; JADE1, KIAA1807)<sup>1-3</sup> is a member of the small JADE family which also includes JADE2 and JADE3 paralogs<sup>3</sup>. So far JADE1 is the most studied member of the JADE family proteins. JADE proteins are encoded by individual genes which are mapped to different chromosomes and designated as PHF17 (JADE1), PHF16 (JADE3), and PHF15 (JADE2) (<http://www.ncbi.nlm.nih.gov>) (Table 1). All three JADE1/2/3 proteins bear in tandem two mid-molecule *Plant Homeodomain (PHD)* zinc fingers. To date, about 90 proteins with one or more PHD zinc finger domains represent a PHD protein family. Amino acid sequences of all JADEs present a high degree of identity in the N-terminal and PHD finger domain regions but are variable in their C-terminal fragments (Fig 1, Identity and Similarity was calculated using “Ident and Sim”<sup>4</sup>). As a result of an alternative splicing, JADE1 mRNA gives rise to two protein products, the full length isoform JADE1L and the truncated version JADE1S which is missing the large C-terminal fragment<sup>3</sup> (Fig 2). To date, this posttranscriptional modification leading to two experimentally detectable protein products appears to be a unique characteristic of JADE1 but not JADE2 or JADE3. The N-terminal fragment of JADE1 presents a degree of homology with the Enhancer of Polycomb Like (<http://pfam.xfam.org/protein/Q6IE81>). JADE1L has two putative protein degradation (PEST) and nuclear localization (NLS) signal peptides. JADE1S has N-terminal PEST

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signal peptide but is missing a putative NLS (Fig 2A). JADE1 proteins are subject to posttranslational modifications via serine and threonine phosphorylation (<http://www.phosphosite.org>). The phosphorylation of several specific functional sites have been demonstrated and proved experimentally by Mass Spectrometry <sup>5-7</sup>.

JADE1 has been mapped to chromosome 4 (4q26-q27). JADE1 is conserved and its orthologs have been found or predicted in most vertebrate and some invertebrate species. Analysis of similarity between human and mouse JADE1 proteins is presented in Fig 2B. Most information about gene structure, conservation, orthologs and paralogs, phylogenetic tree and large scale screening of JADE1 tissue expression are available in several extensive databases (<http://www.genecards.org>; <http://useast.ensembl.org>; see also<sup>3</sup>).

JADE1 is a multifunctional protein and interacts with several protein partners. Except for the function of JADE1S in cytokinesis, most of the insights about other JADE1 functions have originated from the analysis of protein partners of JADE1. As of now the role in histone acetyl transferase complex and the role in the regulation of the cell cycle progression have been studied the most and experimentally supported by different research groups. While the protein product of Von Hippel Lindau gene (pVHL) is the first confirmed protein partner of JADE1, the role in pVHL and beta-catenin signaling pathway is still under investigation and awaits further experimental analysis. Not much is known about the biological role of JADE1 as few *in vivo* studies in human subjects or in mice are available.

The function of JADE2 and JADE3 remains unexplored. There are only two full experimental papers describing the function of JADE2 and JADE3. There is a small number of reports as well as data base entries describing the expression of JADE2 and JADE3. These studies rely on screening and high through put approaches and require further confirmation.

## II. JADE1 Identification and Structure

### II-1. JADE1 Identification

Using the fetal brain cDNA library, Nagase et al. (2001) cloned and sequenced 100 individual cDNAs of which 50 were predicted to have protein products, including clone KIAA1807 which was designated PHF17<sup>1</sup>. The deduced 702-amino acid protein product of KIAA1807 clone was found to be similar to the human zinc finger protein BR140 (BRPF1)<sup>8</sup>. Initial RT-PCR ELISA assays in the same study showed some tissue-specific expression. Based on *in silico* analysis PHF17 was attributed to a category of nucleic acid managing proteins. A study searching for proteins that physically interact with pVHL used the yeast two hybrid pull down approach and, among other candidates, identified a cDNA of a protein which was designated with the name JADE1<sup>2</sup>. The cDNA sequence of JADE1 matched with 5' fragment of the KIAA1807 clone. The deduced 509 amino acid protein product of JADE1 cDNA was described and characterized as a physical partner of the Von Hippel-Lindau protein (pVHL)<sup>8</sup>. Shortly after that, in a genetic screen study searching for genes involved in embryogenesis, the mouse ortholog of JADE1 was identified<sup>3</sup>. The study provided extensive insights about the JADE1 gene and defined the novel JADE family. It also yielded mice with knock out of JADE1.

Based on comparative analysis of the genomic and cDNA sequences a conclusion was made that primary Jade1 transcripts in both humans and mice undergo alternative splicing and polyadenylation, yielding the full length 6 kb mRNA and 3.6 kb mRNA<sup>3</sup>. Two resultant protein products of these transcripts were designated JADE1S for the short isoform (matching KIAA1807<sup>2</sup>) and JADE1L for the full length protein. Moreover, the database analysis revealed two additional homologs (paralogs) of JADE1 which were named JADE2 and JADE3, thus identifying the new JADE subfamily of proteins. Interestingly, JADE3 turned out to be identical to the E9 protein identified in an earlier independent study which suggested a role in apoptosis for PHF16/JADE3/E9 in breast cancer cells<sup>9</sup>.

According to the Human Gene Nomenclature Database ([www.genenames.org/hgnc-searches](http://www.genenames.org/hgnc-searches)) the original symbol for all three genes (that is, PHF15/16/17) was substituted for the name “JADE Family PHD finger 1/2/3”. Therefore, in this review we use the abbreviation JADE1/2/3 for JADE family proteins.

## II-2. PHD Zinc Fingers of JADE1

The PHD zinc finger protein family includes a smaller subfamily characterized by the presence of two PHD zinc fingers: the canonical PHD1 followed by the non-canonical extended PHD2 finger. By virtue of the presence of PHD zinc fingers, all three proteins, JADE1, JADE2, and JADE3, belong to the extended PHD finger protein subfamily. Several other paralogs of JADE1, bearing the PHD finger tandem are known, including BRPF1, BRPF3, BRD1 and others (<http://useast.ensembl.org>). The crystal structure for JADE1 PHD domains has not been solved and its function is still under investigation. Canonical PHD zinc fingers (C4HC3) bind two zinc ions and are present in a number of factors that regulate chromatin-dependent transcription and nucleosome homeostasis<sup>10, 11</sup>. The PHDs are thought to mediate protein-protein interaction and appear to be important for the assembly and activity of multicomponent complexes. Additionally, the PHD fingers might be important for protein stability and folding. Early in vitro studies demonstrated that PHD fingers bind nucleosomes<sup>10</sup>. The canonical PHD finger motif represents a relatively small, stable structure and is distinct from the C3HC4 type RING finger. The extended PHD fingers have similarities with RING domains. According to several studies characterizing individual PHD finger proteins, the canonical PHD domains were able to recognize and bind specific methylated lysine of histone H3 (mostly H3K4me1/2/3) which defined these domains as novel epigenetic histone code readers<sup>12-14</sup>. A number of comprehensive reviews describe structure and properties of PHD fingers in depth<sup>15-18</sup>. Biochemical studies characterizing the binding and specificity of JADE1 PHD zinc fingers towards the N-terminal of histone H3 have been reported<sup>19-21</sup>. According to binding assays, the two PHD fingers of JADE1 appear to bind the N-terminal of histone H3 in a modification-independent manner, although the PHD1 finger alone seems to prefer H3K36me3 histone mark<sup>19, 20</sup>. The crystallization and X-ray analysis of these complexes would need to be done to define the precise JADE1 affinities towards histone marks.

### III. Cellular and Biochemical Function

#### III-1. Histone Acetylation

The first demonstration of nuclear localization and JADE1 function in histone acetylation was reported a decade ago<sup>22</sup>. In cell free assays purified JADE1 protein did not promote acetylation of either soluble or nucleosomal histones. However, in live cell cultures JADE1 dramatically enhanced bulk histone H4 acetylation. This suggested JADE1 interaction with an H4-specific nuclear HAT, which are found only within the MYST family. Indeed, experiments showed that JADE1 physically interacted with TIP60 and via cooperation promoted histone H4 acetylation. The tethering of Gal4-JADE1 fusion protein to Gal4-responsive promoters in co-transfection experiments activated transcription by several fold, suggesting that JADE1 is a transcriptional activator. Thus, JADE1 was identified as a candidate transcription factor which functions in association with a MYST family of HAT<sup>22</sup>. In cultured cells the JADE1-mediated acetylation targets H4K5, H4K12 and most likely H4K8 within histone H4, although these specificities have not been thoroughly confirmed<sup>5, 20, 23</sup>. A correlation with these specificities for JADE1 was also found in vivo in a mice model of tubular epithelial regeneration (discussed in 7)<sup>23</sup>.

The deletion analysis of JADE1 polypeptide in cell free and live cell culture assays revealed the chromatin binding role of the second extended PHD zinc finger domain.<sup>22</sup> Thus, the deletion of PHD2 resulted in a dominant-negative mutant of JADE1. This was evident from functional competition assays, demonstrating that PHD2 is required for global histone H4 acetylation in chromatin context. This was confirmed and dissected further by in vitro binding analysis showing that PHD2 of JADE1 binds histones within a chromatin context irrespective of histone H3 methylation status<sup>20</sup>. These results provided the first experimental evidence for the chromatin-targeting role of JADE1 PHD fingers in histone acetylation and transcription function. The study suggested that by synergizing with an endogenous HAT, JADE1 facilitates acetylation of bulk histone H4 in a nucleosome context. Moreover, results suggested that HAT TIP60 is a candidate for that role.

Consequently, a study characterizing native complexes of Inhibitor of Growth (ING) PHD finger family of proteins found an association of JADE1 with a close homolog of TIP60 and a member of the MYST family, HBO1<sup>24</sup>. According to the study, JADE1S, HAT HBO1, and Eaf6 proteins were present in the ING4/5 pull down complexes, while TIP60, EPC1, Eaf6 and several other proteins were present in the ING3 pull down complex. The striking similarities and identities between TIP60 and HBO1 HAT complexes further supported the role for JADE1 in bulk histone H4 acetylation. Thus, according to protein binding and sequence analysis, JADE1 is a close homolog of EPC1, HBO1 is highly homologous to TIP60, while Eaf6 is the shared member of both MYST complexes<sup>24</sup>. Further investigation of the role for JADE1 in histone acetylation, revealed that JADE1 and HBO1 interactions have striking structural and functional similarities with TIP60<sup>22, 25</sup>. Thus, similar to TIP60, JADE1 and HBO1 mutually stabilize each other<sup>25</sup>. Moreover, similar to TIP60, the ability of HBO1 to acetylate histone H4 in a chromatin context was dramatically enhanced by JADE1 and this required an intact PHD2 finger of JADE1. In contrast, the PHD protein ING4/5 did not potentiate histone acetylation activities of HBO1 or HBO1-JADE1 complex in either

assay<sup>25</sup>. These interactions with two HATs raise a question: What are the mechanisms that ensure specificities of interactions between homologs in the cellular context. Sharing partners and a combinatorial mode of interaction has been described for other chromatin-associated complexes. It is possible that by switching the two known HAT partners JADE1 might perform distinct functions in different cellular compartments during the cell cycle (discussed in III-2).

Although nuclear JADE1 complex composition has been investigated and the role in HAT-dependent gene transcription proposed, neither the specific transcription targets nor the transcription function of JADE1 have been identified<sup>22</sup>. Potential transcription targets of JADE1 have been suggested from experiments using screening approaches, but so far not confirmed<sup>20, 21</sup>.

Evidence for biochemical, cellular, and in vivo differences between the two JADE1 isoforms have been demonstrated but remain puzzling (discussed throughout the review). For example, structurally, JADE1S lacks the C-terminal fragment and hence is incapable of binding ING4/5, which are well characterized tumor suppressors and PHD zinc finger proteins<sup>25</sup>. As a result, at a minimum, JADE1 isoforms assemble two different complexes, JADE1L-HBO1-ING4/5 and JADE1S-HBO1 complex<sup>25</sup>. A small less characterized protein Eaf6 might be another component of JADE1 complexes<sup>21</sup>. JADE1L complexes with TIP60 have never been studied.

## III-2. Cell Cycle Regulation

**III-2-1. DNA Replication**—It has been reported that lysine acetylation of the N-terminal tails of bulk histone H4 correlates with DNA synthesis, suggesting a role in cell proliferation<sup>26-30</sup>. Importantly, JADE1 is required for the acetylation of bulk histone H4 in cultured cells<sup>22, 23, 25</sup>. In search of the cellular function of JADE1, studies were initiated to examine the JADE1 role in cell cycle progression<sup>5, 6, 23</sup>. While these studies established JADE1 function in cell cycle regulation, the mechanisms of action, the stages involved and, most importantly, the specific contribution of JADE1L and JADE1S isoforms are yet to be identified. As evident from the results, the two isoforms of JADE1 appear to accomplish different functions during epithelial cell division.

The following experimental observations support the cell cycle role for JADE1 in conjunction with the HBO1-mediated pathway<sup>5, 23, 31</sup>. The depletion of JADE1 proteins by RNA interference (siRNA) resulted in slower rates of DNA synthesis in cell cycle-synchronized epithelial cell lines and primary fibroblast cell cultures<sup>23</sup>. The siRNA reagent used in these experiments targeted both isoforms of JADE1. In addition, the depletion of JADE1 proteins resulted in decreased levels of the total and chromatin-bound HBO1, which nicely correlated with previously described effects of JADE1-HBO1 mutual upregulation<sup>23, 25</sup>. In dividing synchronized cultures, JADE1 depletion prevented time-dependent chromatin recruitment of MCM7<sup>23</sup>. Agreeing with these results, JADE1L overexpression in asynchronously dividing cell cultures increased total chromatin-bound MCM3 protein<sup>32</sup>. The effect of JADE1 depletion on MCM7 chromatin recruitment recapitulates that of HBO1 protein depletion originally reported by Mitch Smith and colleagues<sup>33</sup> and suggests a role for JADE1 in the HBO1 pathway. The JADE1 role in DNA licensing has

been suggested but not proven. Based on data available so far it is more likely that JADE1 functions in DNA synthesis (S-phase) rather than replication licensing (G1-phase).

A recent report describing novel non-coding RNA lncRNA-JADE supports the role for JADE1 in DNA synthesis linked to histone H4 acetylation<sup>34</sup>. In addition, the study demonstrates that lncRNA-JADE provides a functional link between the DNA damage response (DDR) and bulk histone H4 acetylation. Knocking down of lncRNA-JADE in cultured cells increased cells sensitivity to DNA damaging drugs. In the mice tumor xenograft model the down regulation of lncRNA-JADE resulted in inhibition of xenograft mammary tumor growth. In a pilot human study, higher levels of lncRNA-JADE as well as JADE1 protein were detected in breast cancer tissues compared to normal tissues and the higher levels of JADE1 protein inversely correlated with survival rates of patients with breast cancer. The study suggests that lncRNA-JADE might contribute to breast tumorigenesis and that JADE1 protein mediates at least part of this effect<sup>34</sup>. It is unclear whether shRNA used in this study targeted JADE1S, JADE1L or both isoforms. In light of the recent evidence of differential effects of the two JADE isoforms<sup>5, 6</sup>, it would be very interesting to assess the contribution of individual proteins to DDR and tumor growth.

**III-2-2. Cytokinesis**—Recently, a novel and rather unexpected function of JADE1 was reported in the regulation of cytokinesis of the epithelial cell cycle which was specific to the small isoform, JADE1S<sup>6</sup>. The first hint that JADE1 operates between G2/M and G1 of the cell cycle came from the previous report which identified and characterized cell cycle-dependent dynamic properties of JADE1<sup>5, 31</sup>. According to the study, during the late G2 phase, JADE1S undergoes phosphorylation linked to its dissociation from chromatin into the cytoplasm. Mass Spectral analysis identified that a total of six individual amino acid residues are phosphorylated by a mitotic kinase, including two novel residues<sup>5</sup>. Based on pharmacological analysis, JADE1 phosphorylation and compartmentalization is regulated by Aurora A and Aurora B pathways<sup>5, 6</sup>. Other kinases have been reported and may play a role<sup>7, 35</sup>. Upon completion of mitosis around the telophase, the main pool of the JADE1S protein undergoes dephosphorylation and re-associates with apparently condensing chromatin inside the reformed nuclei<sup>5</sup>. Strikingly, a minor pool of JADE1S follows the cleavage furrow and subsequently appears in the midbody of the cytokinetic bridge<sup>6</sup>. Interestingly, only JADE1S, but not JADE1L or HBO1 were found in the midbody of the cells undergoing cytokinesis. The cell cycle-dependent regulation of JADE1S suggested a role in G2/M to G1 transition, which includes cytokinesis and final abscission stages of the cell division<sup>6, 36</sup>.

Cytokinesis orchestrates several steps of cell division, including chromosome segregation, cell membrane furrowing, actin cytoskeleton remodeling, and the assembly of connecting bridge and midbody. The final abscission occurs near the midbody which may take up to 2 hours and has been actively studied in recent years. Cytokinesis and final abscission are tightly controlled by regulatory protein complexes, including checkpoint proteins. The number of breakthrough experimental studies and excellent reviews that cover the topic of cytokinesis control has been growing over the past decade<sup>37-41</sup>.



The evidence for the JADE1 role in cytokinesis came from experiments using several functional approaches and cell culture models<sup>6</sup>. A set of experiments using DNA profiling by FACS showed that JADE1S depletion facilitated rates of G1-cells accumulation in synchronously dividing HeLa cells. Further, JADE1S protein depletion in asynchronously dividing cells decreased the proportion of cytokinetic cells. These results show that JADE1 negatively controls cytokinesis, presumably by contributing to cytokinesis delay. Moreover, JADE1 down-regulation caused increased proportion of multi-nuclear cells, which is indicative of premature and failed cytokinesis. In contrast, moderate overexpression of JADE1S increased the number of cytokinetic cells in a time- and dose- dependent manner, indicating cytokinetic delay. The progression of mitosis and cytokinesis is controlled by the activities of checkpoint master kinases Aurora A and B. Pharmacological inhibition of NoCut checkpoint kinase Aurora B resulted in the release of JADE1S-mediated cytokinetic delay and the progression of abscission. Thus, JADE1S is likely to regulate cytokinesis at the abscission checkpoint control<sup>6, 36</sup>.

**III-2-3. JADE1L versus JADE1S**—Interestingly that JADE1S but not JADE1L or HBO1 was localized to centrosomes of the dividing cells. These data further highlighted differences between JADE1 isoforms. None of these three proteins was found in cilia. Centrosomal localization of JADE1 was also reported earlier in another study. However, in this study the antibody used is of unknown specificity and therefore does not allow discrimination between JADE1 isoforms<sup>35</sup>. Centrosome signaling contributes to the definition of cell shape, motility, orientation, polarity, division plane and to the fidelity of sister chromosome separation during mitosis and cytokinesis<sup>42, 43</sup>. During the S phase of the cell cycle centrosomes undergo duplication. It is notable that centrosome duplication is tightly controlled by cdk-dependent kinases and that similar to DNA replication licensing, has to occur once and only once during the cell cycle. It has been suggested that as part of the coordination mechanism, shared pathways and factors would control spatial and temporal regulation of centrosome duplication, DNA replication, mitosis and cytokinesis<sup>42, 43</sup>. Based on data available so far, during the cell cycle, JADE1 is involved in a puzzling set of alternative activities that are hard to reconcile. The possible explanation is that the two JADE1 isoforms might be involved in spatial-temporal coordination of the DNA synthesis and cytokinesis and perhaps direct transcription regulation of cell cycle factors. In this case the phosphorylation of specific amino acid residues by cell cycle kinases (cdk's, Aurora A and B kinases, others) would target JADE1S and JADE1L to the appropriate cellular compartments to enable their function in a given phase of the cell cycle progression (see schematics of summaries in Fig 3 and Table 2).

In addition to JADE1S, several transcription factors, histone and DNA modifiers with predominantly nuclear localization such as Brd4 and WDR5, have been found to regulate cytokinesis and abscission<sup>44, 45</sup>. Recently, it has been reported that the novel Abscission/NoCut Checkpoint Regulator (ANCHR) protein functions in Aurora-B-dependent abscission checkpoint control in HeLa cells<sup>46</sup>. Interestingly, ANCHR polypeptide includes FYVE and RING finger domains, while PHD2 finger of JADE1S may have structural similarities with the RING finger. PHD fingers of JADE1S are required for chromatin association and are candidate epigenetic code readers<sup>19, 20, 22, 25</sup>. It would be interesting to find out whether

PHD zinc fingers play a role in the novel JADE1S activities during cytokinesis and potentially abscission.

It has been shown that similar to phosphorylation, lysine acetylation of proteins is involved in centrosome-mediated cytokinesis control<sup>47-49</sup>. The function of JADE1S in cytokinesis raises a question whether this activity is dependent on protein acetylation and interactions with HATs (HBO1, TIP60, others). We have, thus far, not detected HBO1 in the midbody and centrosomes and assessments of TIP60 have not been completed yet<sup>6</sup>. Alternatively, JADE1 protein might operate in complex with other identified or as yet unknown protein partners.

### III-3. JADE1 and pVHL

The von Hippel–Lindau tumor suppressor (pVHL) is a protein product of the human VHL gene which is mutated in von Hippel–Lindau hereditary disease and in the majority of sporadic clear cell renal carcinomas<sup>50-54</sup>. The pVHL is the first protein partner of JADE1S isoform which was identified in 2002<sup>2</sup>. Consequently, a few more studies characterized JADE1-pVHL interactions and suggested a role in apoptosis and tumor suppression<sup>55-57</sup>.

The properties and function of pVHL have been investigated for many decades and an extensive literature is available. One of the better known functions of the pVHL protein is the contribution to the protein ubiquitination and degradation pathway. As a component of the ubiquitin ligase E3 complex, pVHL binds and targets several factors, including HIF1a and HIF2a for proteolytic degradation<sup>53</sup>. An elegant study unraveling the molecular mechanism of oxygen sensing and describing pVHL-dependent HIF1a activation by hypoxia was reported over a decade ago<sup>58</sup>. At least two isoforms of pVHL protein are known: the 19 kDa short and 30 kDa long isoforms. Both isoforms have been intensely studied and the link of naturally occurring pVHL mutations to cancers established. While cancer associated mutations affect ubiquitination and proteasome targeting activities of pVHL, the mere disruption of this function does not fully explain the mechanisms of pVHL-mediated cancer pathogenesis and sporadic renal cancer disease - a phenomenon which has been challenging scientists in the field. Defects with other pVHL cellular activities and pathways have been proposed and considered<sup>59</sup>. In a study searching for new pVHL partners, JADE1S has been identified by use of the yeast two hybrid screening analysis<sup>2</sup>. The pVHL-JADE1S physical interaction was further confirmed biochemically. Interestingly co-transfection of pVHL increased protein half-life and JADE1S protein abundance, suggesting a potential positive relationship<sup>2</sup>. Certain pVHL cancer-derived truncations but not point mutations diminished the pVHL-JADE1 stabilization function, suggesting a link to cancer<sup>57</sup>.

Although some aspects of pVHL-JADE1 interactions have been characterized, the molecular mechanisms and more importantly physiological role of such interactions remain unclear. One study reported that JADE1S has intrinsic ubiquitin-ligase activity and is capable of binding and ubiquitination of beta-catenin<sup>55</sup>. Based on that study, a model has been proposed that pVHL regulates beta-catenin abundance through JADE1 and that PHD zinc fingers are required for this activity. Additional experimental proof and a follow up study would need to support this possibility.



### III-4. Is There Function in Apoptosis?

The role of JADE1S in apoptosis was proposed in early studies which were often reliant on overexpression approaches<sup>2, 21, 55-57</sup>. Due to the nature of the cell cycle the ectopic expression of high, non-physiological levels of cell cycle regulatory proteins often results in deleterious cellular effects. In various cell cultures, we have repeatedly observed that the introduction of JADE1 protein into the growing cells by conventional methods renders toxic consequences and morphological changes that do not resemble apoptosis<sup>6</sup>. Transient transfections of JADE1S using cDNA in quantities suggested by the manufacturers typically results in apparently dying cells with abnormal shapes and large, multi-lobular nuclei<sup>6</sup>. This diminishes the apparent transfection efficiency, most likely due to cell death and negative self-selection. Several earlier attempts to establish dependable cell lines stably expressing JADE1S protein have failed. In light of current findings, the cell death that has been observed after JADE1S overload may have been caused by the prolonged NoCut and stalled cytokinesis rather than direct transcription activation of apoptotic drivers by JADE1S. While the role in signaling of apoptosis is not excluded, these studies and their interpretations may need to be re-visited. A reliable approach to investigate JADE1S cellular function, including proposed apoptosis, must include controlled isoform-specific knockdown of JADE1 or ectopic expression of physiologically relevant protein quantities. At this point such experiments examining JADE1 apoptosis-signaling function have not been done.

## IV. Biological Function

### IV-1. Murine Models

A possible in vivo role of JADE1 in epithelial cell proliferation was addressed in a murine model of acute kidney injury and regeneration<sup>5, 23</sup>. The results of the study strongly suggest a role for JADE1 in the tubular cell proliferation regeneration phase as well as differential roles for JADE1S and JADE1L. Expression patterns and dynamics of HBO1-JADE1S/L were examined in regenerating tubular epithelial cells<sup>23</sup>. Ischemia and reperfusion injury resulted in an initial decrease in JADE1S, JADE1L, and HBO1 protein levels, which returned to the baseline during renal recovery. Expression levels of HBO1 and JADE1S recovered as cell the proliferation rate reached maximum, whereas JADE1L recovered after bulk proliferation had diminished. The temporal expression of JADE1 correlated with the acetylation of histone H4 on lysines 5 and 12, but not with acetylation of histone H3 on lysine 14, suggesting that in vivo the JADE1-HBO1 complex specifically marks H4 during epithelial cell proliferation. The results of the study implicate JADE1-HBO1 complex in acute kidney injury and yet again suggest distinct roles for JADE1 isoforms during epithelial cell recovery<sup>23</sup>.

The biological role of JADE1 has not been established. There is a limited number of publications that attempt to address this question using adult and embryonic mice models. The most informative remains the earlier study which identified the mouse ortholog of human JADE1, *Jade1*, and described its expression during mouse embryogenesis<sup>3</sup>. Searching for developmentally regulated genes expressed in the primitive streak and tail bud, the authors of the study used the gene trap screen and identified mouse *Jade1* as the gene strongly regulated during embryogenesis. Insertion of the vector occurred in the third intron

of the mJade1 gene, leading to the production of a 47-amino-acid truncated protein. The gene trap insertional mutation resulted in JADE1-beta-galactosidase reporter fusion product and JADE1 null allele. While the homozygotes for the gene trap integration did not produce a strong developmental phenotype, the fusion product allowed detailed and precise examination of Jade1 gene spatial-temporal expression in mouse embryonic cells and tissues of the developing embryo up to 15.5-d.p.c. In addition, the study reports experimental and in silico comparative analysis of JADE1 mRNA transcripts, JADE1 gene structure, and JADE1 protein orthologs from mouse, human and zebra fish<sup>3</sup>.

According to the study, strong mJade1 expression was detected in extraembryonic ectoderm and trophoblast, which are placental components important for vasculogenesis, as well as in sites enriched with multipotent or tissue-specific progenitors, including neural progenitors<sup>3</sup>. The dynamics of mJade1-b-Gal expression in these areas indicates that JADE1 may be involved in determination and elongation of the anterior posterior axis, which was one of the important suggestions of the study<sup>3</sup>. The potential role for JADE1 in the renewal of embryonic stem cells and embryonal carcinoma cells was suggested in another screening study which used cultured cell models. According to that study, the activation of stem cell transcription factor OCT4 pathway upregulated JADE1 gene expression along with stem cell factors NANOG, PHC1, USP44 and SOX2<sup>60</sup>. It would be interesting to investigate whether there is a biological relevance between JADE1 functions in the cell cycle and JADE1 activities communicated in these two studies.

#### IV-2. Human and Diseases

The potential role of JADE1 in human disease is under investigation. As a novel cell cycle regulator and candidate transcription factor, JADE1 represents an interesting pathway to explore the pathogenesis of epithelial cancers and regenerative disorders. In fact, two of the JADE1 partners and several proteins closely related to JADE1 are mutated in cancers and are tumor suppressors<sup>50, 61</sup>. An interesting recent study was set out to search for submicroscopic genetic changes in myelofibrosis which is a bone marrow cancer<sup>62</sup>. The study identified seven novel deletions and translocations in five out of a total of 15 patients with primary myelofibrosis. JADE1 along with the adjacent gene called Sodium channel and clathrin linker 1 (SCLT1) were found to be significantly modified. The resultant mutated JADE1 gene has deletions of intron 5-6 and exons 6-11 which would result in mutated JADE1 missing a large chunk of protein starting from the PHD zinc finger. The relevance to pathogenesis is under investigation.

In a handful of pilot studies JADE1 expression was examined in colon cancers and renal carcinomas. The results of these studies do not always reconcile perhaps due to the fact that they were generated predominantly from the histochemical analysis of tumor specimens using JADE1 antibody with uncharacterized specificities towards JADE1 in general, and JADE1S or JADE1L in particular<sup>63, 64</sup>. Results of a study using in silico microarray algorithm analysis show that PHF17 mRNA may play a role in the development of pancreatic cancer<sup>65</sup>. While promising, these lines of investigation require further controls and additional assessments to warrant any conclusions.

### IV-3. Challenges and Future Directions

The understanding of the mechanism of cell cycle regulation by JADE1 is a necessary prerequisite for the clarification of the JADE1 cellular and biological function. The puzzling relationship between JADE1S and JADE1L isoforms and their specific functions appears to be especially important. Major points to tackle would be centrosome- and midbody-associated activities of JADE1 in cytokinesis as well as chromatin-dependent regulation of DNA synthesis. JADE1 transcription activities and targets remain to be determined. The cell cycle activities of JADE1, including phosphorylation and shuttling, HAT-dependent epigenetic control of DNA synthesis, and cytokinesis control presumably via NoCut final abscission check point suggest the *in vivo* role of JADE1 in epithelial tissue regeneration, homeostasis and in proliferative diseases. The role of cytokinesis control in cancer pathogenesis has been recently appreciated and is under active investigation. Therefore, JADE1 might represent one of the new key pharmacological targets for anticancer drug development. Use of various mice models, including JADE1 knock out will be instrumental to address these questions. Controlled human studies examining potential disease-relevant mutations of the JADE1 gene and the expression of JADE1 isoform-specific mRNA and protein will help to determine their role in cancer. Very little is known about the two other members of the JADE family, JADE2 and JADE3. Except for the variable C-terminal tail, the amino acid sequences of JADE family proteins are highly homologous. Hence, the similarity suggests an interchangeable compensatory role, while the variability could define tissue distribution or specific pathways involved. Future investigations of JADE2 and JADE3 will facilitate our understanding of the cellular and biological role of JADE1 as well as the JADE family.

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### Abbreviations

<b>JADE1/2/3</b>	Gene for Apoptosis and Differentiation 1/2/3
<b>PHF15/16/17</b>	Plant Homeodomain Factor 15/16/17
<b>PHD</b>	Plant Homeo Domain
<b>RING finger</b>	<u>R</u> eally <u>I</u> nteresting <u>N</u> ew <u>G</u> ene
<b>pVHL</b>	Von Hippel Lindau protein
<b>MYST family</b>	the name of the HAT family is abbreviated from the names of the original members ( <u>M</u> oz, <u>Y</u> bf2 (Sas3),

Sas2, and Tip60). Other members of MYST family are HBO1, MOF, MORF and Esa1.

<b>HBO1</b>	<u>HAT Binding Origin of Replication Complex</u> <u>1</u>
<b>TIP60</b>	HIV1 <u>Tat</u> interacting protein
<b>ING4, ING5</b>	<u>Inhibitor of Growth</u> <u>4</u> and <u>5</u>
<b>MCM7</b>	<u>Mini-chromosome maintenance</u> protein <u>7</u>
<b>BRD4</b>	<u>Bromodomain-containing</u> protein <u>4</u>
<b>BR140/BRPF1</b>	<u>bromodomain and PHD finger-containing</u> protein <u>1</u>

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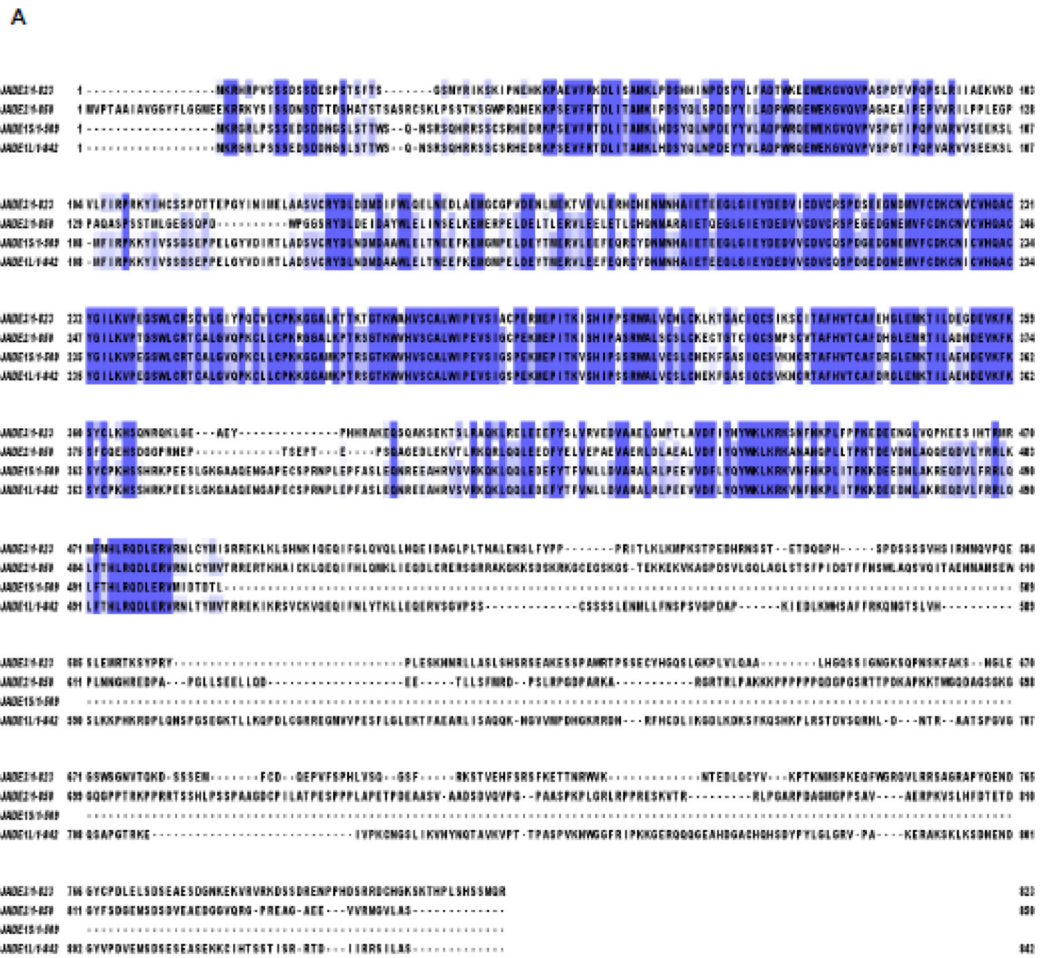
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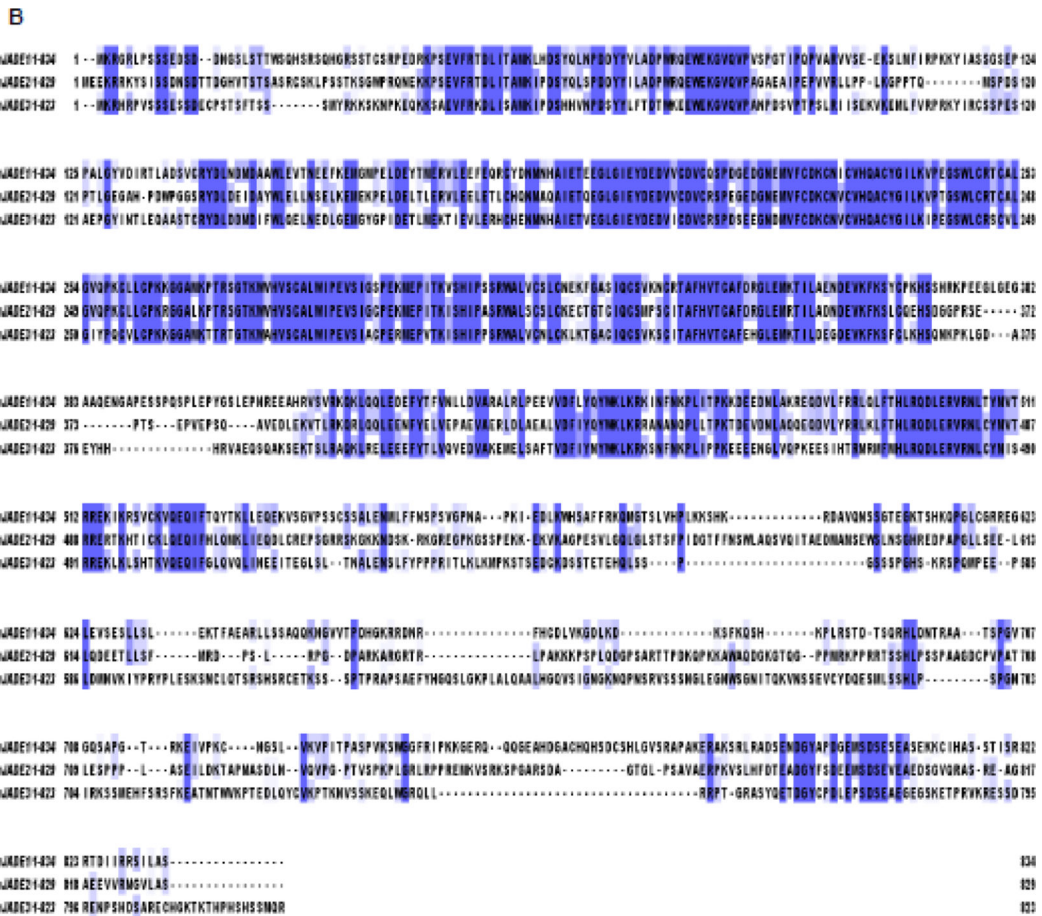
### Highlights

- PHD zinc finger proteins JADE1, JADE2, and JADE3 make up a small family of proteins.
- JADE1 facilitates bulk histone H4 acetylation via interaction with an endogenous HAT.
- JADE1S and JADE1L isoforms of JADE1 appear to play different cellular roles.
- JADE1 proteins directly regulate cell cycle progression.
- JADE1S is a negative regulator of cytokinesis and possibly the NoCut checkpoint.



Results for JADE1L vs JADE1: Alignment length: 842 Identical residues: 503 Similar residues: 1 Percent identity: 59.74 Percent similarity: 59.86	Results for JADE1L vs JADE2: Alignment length: 864 Identical residues: 408 Similar residues: 95 Percent identity: 47.22 Percent similarity: 58.22	Results for JADE1L vs JADE3: Alignment length: 894 Identical residues: 368 Similar residues: 92 Percent identity: 41.16 Percent similarity: 51.45
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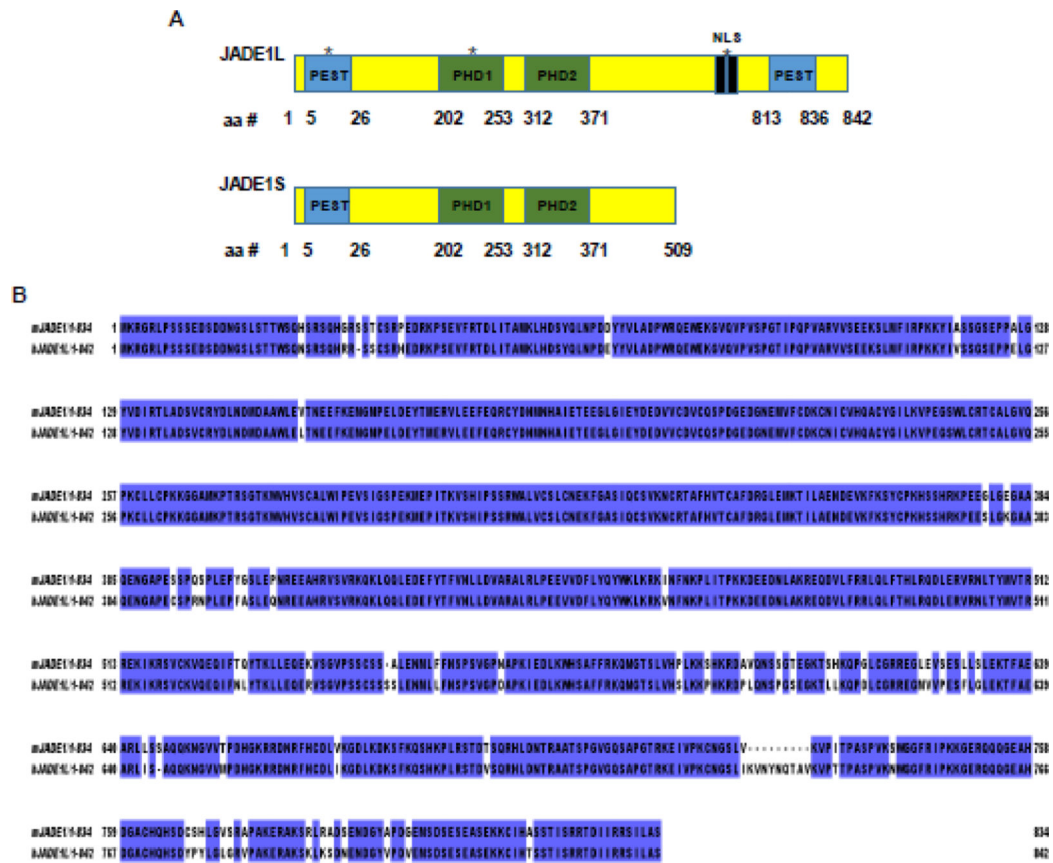
Figure 0001



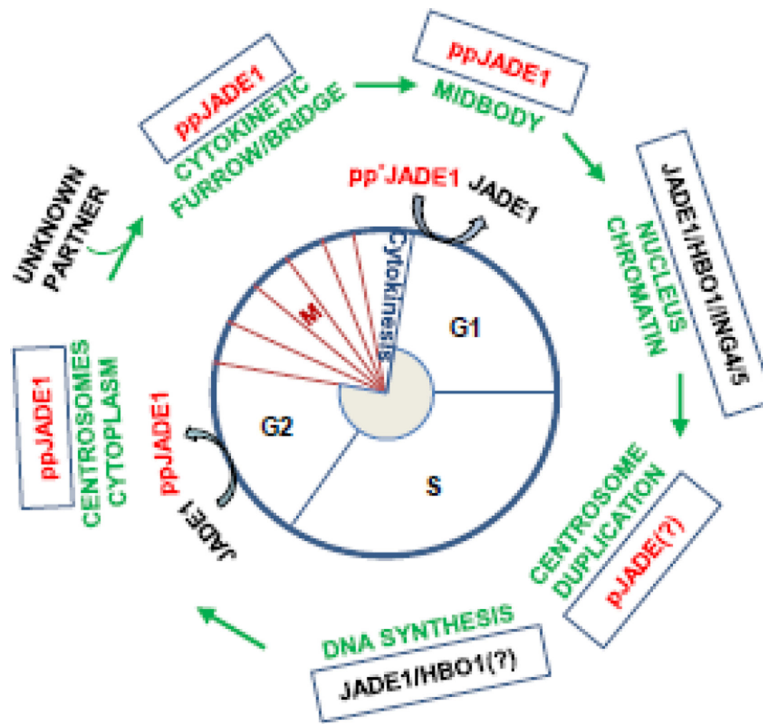
Results for mJADE1 vs mJADE2: Alignment length: 882 Identical residues: 417 Similar residues: 88 Percent identity: 47.28 Percent similarity: 57.26	Results for mJADE1 vs mJADE3: Alignment length: 911 Identical residues: 357 Similar residues: 106 Percent identity: 39.19 Percent similarity: 50.82	Results for mJADE2 vs mJADE3: Alignment length: 909 Identical residues: 333 Similar residues: 105 Percent identity: 36.63 Percent similarity: 48.18
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**Figure 0002**  
**Figure 1. Sequence alignment of JADE family PHD finger proteins**  
**A, Human. B, Mouse.** Protein sequences were obtained from UniProt, and aligned using Clustal Omega (<http://www.ebi.ac.uk/Tools/msa/clustalo/>). Identity and Similarity was calculated using “Ident and Sim” (see the text for reference).

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**Figure 2.**  
A, The domains of the human JADE1 protein isoforms. \*PHD1 and PHD2 - Cys4HisCys3 plant homeo domain; NLS – nuclear localization signal; PEST - protein degradation amino acid sequence (proline, glutamic acid, serine, and threonine). B, Alignment of amino acid sequences of mouse and human JADE1. Sequences from the Uniprot database (Q6IE81-1 and Q6ZPI0-1) were aligned with Jalview software.



**Figure 3. JADE1 function in the cell cycle**  
 For details see the text. \* indicates phosphorylation



Table 1

The JADE family PHD finger genes and proteins from human and mouse

Gene symbol	JADE1 human	Jade1 mouse	JADE2 human	Jade2 mouse	JADE3 human	Jade3 mouse
Chromosome number	4	3	5	11	X	X
Location	128,809,623-128,875,224	41,555,734-41,616,864	134,524,312-134,583,230	51,813,455-51,857,653	20,425,688-20,519,939	20,425,688-20,519,939
Protein product(s)-length	JADE1L-842 aa; 95.5K Da; JADE1S-509 aa; 58.4 KDa	Jade1L-834 aa; 93.9 Kda; Jade1S-510 aa; 58.1 KDa	JADE2-790 aa; 87.5 KDa	Jade2-829 aa; 92.2 KDa	JADE3-823 aa; 93.8 KDa	Jade3-823 aa; 93.5 KDa
Ensemble gene ID	ENSG00000077684	ENSMUSG00000025764	ENSG00000043143	ENSMUSG00000020387	ENSG000000102221	ENSMUSG00000037315
UniProt ID	Q6IE81-1 (JADE1L) Q6IE81-3 (JADE1S)	Q6ZPI0-1 (Jade1L) Q6ZPI0-2 (Jade1S)	Q9NQC1	Q6ZQF7	Q9Z6I3	Q6IE82

**Table 2**

Function and regulation of JADE1 protein isoforms in epithelial cells and tissues.

JADE1 isoform	G1/S		G2/M		Cytokinesis	
	JADE1L	JADE1S	ppJADE1L <sup>**</sup>	ppJADE1S <sup>**</sup>	JADE1L	pJADE1S <sup>**</sup>
<b>JADE1 subcellular localization</b>	Chromatin	Chromatin Centrosome	Cytoplasm	Cytoplasm Centrosome	Chromatin	Cleavage furrow, Midbody
<b>JADE1 activities</b>	Histone H4 bulk acetylation, homeostasis. DNA synthesis. Replication licensing(? <sup>*</sup> ). Transcription(? <sup>*</sup> ).		JADE1S is phosphorylated by Aurora A pathway. Bulk of pJADE1 <sup>**</sup> is removed from chromatin to allow chromosome condensation(? <sup>*</sup> ).		Specific fraction of pJADE1S <sup>**</sup> mediates NoCut in Aurora B pathway. Dephosphorylated JADE1S/L re-associates with relaxing chromatin	
<b>JADE1 dysregulation: predicted outcomes</b>	DNA damage, amplification, impaired tissue homeostasis, degeneration, or cancer		Premature or delayed cytokinesis, cytokinesis failure with following aneuploidy, polyploidy, gene mutations, cell death or transformation. Impaired tissue homeostasis, degeneration, or cancer			

The hypothetical scheme summarizes current literature about cellular activities of JADE1 protein isoform activities during the cell division.

\* predicted

\*\* phosphorylated form of JADE1 proteins