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Emerging role of DYRK family protein kinases as regulators of protein stability in cell cycle control

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Dual-specificity tyrosine phosphorylation-regulated kinases (DYRKs) constitute an evolutionarily conserved family of protein kinases with key roles in the control of cell proliferation and differentiation. Members of the DYRK family phosphorylate many substrates, including critical regulators of the cell cycle. A recent report revealed that human DYRK2 acts as a negative regulator of G_1/S transition by phosphorylating c-Jun and c-Myc, thereby inducing ubiquitination-mediated degradation. Other DYRKs also function as cell cycle regulators by modulating the turnover of their target proteins. DYRK1B can induce reversible cell arrest in a quiescent G_0 state by targeting cyclin D1 for proteasomal degradation and stabilizing $p27^{Kip1}$. The DYRK2 ortholog of *C. elegans*, MBK-2, triggers the proteasomal destruction of oocyte proteins after meiosis to allow the mitotic divisions in embryo development. This review summarizes the accumulating results that provide evidence for a general role of DYRKs in the regulation of protein stability.

Keywords: DYRK1A, DYRK1B, DYRK2, HIPK2, MBK-2, Yak1, phosphodegron, ubiquitin cyclin D1, $p27^{Kip1}$

Abbreviations: APC, anaphase-promoting complex; DCAF, DDB1 and CUL4-associated factor; DYRK, dual-specificity tyrosine phosphorylation-regulated kinase; HIPK, homeodomain-interacting protein kinase; REST, RE1 silencing transcription factor; SCF, E3 ligase complex containing Skp, cullin and F-box protein; UPS, ubiquitin proteasome system

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DYRK Family Protein Kinases

Kinases of the DYRK family were discovered as key regulators of cell growth and differentiation in genetically tractable organisms such as budding yeast (Yak1), fission yeast (Pom1), *Dictyostelium* (YakA) and Drosophila (MNB).[1,2] Human DYRK1A was discovered as the product of a gene localized in the Down syndrome critical region on chromosome 21.[3] DYRK1A has been most extensively studied among the mammalian DYRKs, because its overexpression in trisomy 21 is believed to contribute to the neuropathological traits of Down syndrome.[4,5] DYRK1A and the closely related DYRK1B (also known as MIRK) have also been characterized as negative regulators of the cell cycle that mediate cell survival and promote the switch to a quiescent state or differentiation.[6-9] DYRK2 can induce apoptosis upon genotoxic stress by phosphorylating p53.[10] Although members of the DYRK family are engaged in multiple and diverse regulatory processes in different experimental systems, a recurrent theme of their functions in mammalian cells as well as in yeasts, *C. elegans* and Dictyostelium is their role as key regulators of different checkpoints in the cell cycle.

Target Proteins of DYRKs

An increasing number of substrates and functions in signal transduction pathways is being reported for DYRKs from different organisms. Downstream effects mediated by target proteins of DYRKs include the increased activity of transcription factors, the modulation of subcellular protein distribution and the regulation of enzyme activity. Recent reviews provide an excellent overview of the biochemical properties and the currently known substrates of DYRK1A as well as the other kinases of the DYRK family.[2,11] One characteristic feature of several DYRK kinases is their function as priming kinases, meaning that the phosphorylation of a given residue by a DYRK is prerequisite for the subsequent phosphorylation of a different residue by

another protein kinase (GSK3 or PLK).[2] Here we want to call attention to another effect common to several members of the DYRK family, namely the control of protein stability. This function of DYRKs has been brought into the limelight by a new report from Taira et al.,[12] who identified DYRK2 as the kinase controlling c-Jun and c-Myc degradation at the G_1/S boundary. This finding adds to accumulating evidence that members of the DYRK family from diverse organisms modulate the turnover of target proteins either by inducing degradation by the ubiquitin-proteasome system (UPS), or by stabilizing short-lived proteins.

This review summarizes the current knowledge that DYRKs function in the regulation of protein stability. Emphasis is placed on proteins involved in cell cycle control, and the scope is limited to the members of the DYRK subfamily. It should be noticed, however, that the closely related homeodomain-interacting protein kinase 2 (HIPK2) has also been reported to regulate the turnover of some target proteins. Exemplary data for HIPK2 are included in **Table 1**, which lists the published evidence for the regulation of protein turnover by the DYRK family.

DYRK2 Initiates Protein Degradation via the UPS

Two major types of E3 ubiquitin ligase complexes catalyze the phase-specific ubiquitination of proteins in the cell cycle, the anaphase-promoting complex (APC) multisubunit E3 ligase and the SCF form of E3 ligases. SCF E3 ligases belong to the major group of cullin-based E3 ligases, which consist of four kinds of protein subunits: an adaptor protein (Skp1 in SCF), a scaffold protein termed a cullin (CUL1 in SCF), an E2-recruiting subunit (Roc1/Rbx1/Hrt1) and a substrate receptor (one of about 70 F-box proteins in SCF).[13] Phosphorylation-dependent protein degradation is a common mechanism for regulating protein stability in a cell cycle-dependent or stimulus-dependent manner. Kinases create phosphodegron motifs in the substrate proteins, which are then recognized by F-box proteins and ubiquitinated by E3 ligase complexes.

The recent study of Taira et al.[12] reveals interesting details about the molecular mechanism by which DYRK2 regulates the turnover of c-Myc and c-Jun. Many tumor cells depend on high levels of c-Jun and c-Myc to enter S phase. Cellular levels of these oncogenic transcription factors are controlled by proteasomal degradation, which is initiated upon phosphorylation by GSK3β. DYRK2 has now been identified as the priming kinase for the phosphorylation of c-Jun and c-Myc by GSK3β, meaning that phosphorylation of the substrate at the P+4 position by DYRK2 is required for substrate recognition by GSK3β.[12] The subsequent phosphorylation of the P0 residue by GSK3β creates a phosphodegron required for the binding of an SCF E3 ligase complex containing the F-box protein Fbw7, eventually resulting in the polyubiquitination and ensuing proteasomal degradation of c-Jun/c-Myc (**Fig. 1**). DYRK2 was shown to play a key role in this chain of events, since the knockdown of DYRK2 in human cancer cells shortened the G_1 phase and accelerated cell proliferation due to the escape of c-Jun and c-Myc from ubiquitination-mediated degradation.

DYRK2 has been reported to function as a scaffold for the assembly of an E3 ligase complex with a protein composition similar to cullin4A-RING E3 ubiquitin ligase (CRL4) but lacking the cullin protein.[14] This complex was shown to catalyze the phosphorylation and subsequent ubiquitination of katanin p60, a microtubule-severing enzyme with an important role in the mitotic reorganization of spindle microtubules. The authors have proposed that the catalytic domain of DYRK2 harbors a KELCH motif, which is a feature of several proteins acting as E3 ligase adaptors for specific substrates. Functional KELCH motifs allow for many substitutions and cannot unambiguously be identified by the sequence alone. However, the organization as a twisted β-sheet motif arranged in a propeller-like structure is invariable.[15] The existence of a functional KELCH repeat at the position suggested (amino acid residues 390–433) must be excluded, because this region of DYRK2, as in all protein kinases, is made up by α helices (PDB accession 3KVW).

Another target of DYRK2 is the transcription factor GLI2, a primary downstream effector of the hedgehog pathway with a proliferative effect in many tumors.[16] Phosphorylation by DYRK2 induces the degradation of GLI2 by the UPS.[17] A previous study had identified a phosphodegron for recognition by the $SCF^{\beta\text{-}TrCP2}$ E3 ligase and proposed GSK3 as the relevant kinase.[18] However, there is no evidence for a priming function of DYRK2, and it is also not known whether the DYRK2 phosphorylation sites directly control binding of an E3 ligase. Further research is required to reveal the individual or synergistic roles of DYRK2 and GSK3 in the regulation of GLI2 turnover.

It is remarkable that the ortholog of DYRK2 in *C. elegans,* MBK-2, has also a critical function in the control of protein degradation. Due to the availability of both genetic and cell biological methods, the role of MBK-2 in *C. elegans* embryogenesis has been characterized in great molecular and functional detail. MBK-2 is activated in zygotes at meiosis II and phosphorylates three proteins, MEI-1, OMA-1 and OMA-2, promoting their timely degradation to allow oocyte-to-embryo transition.[19-21] MBK-2 acts as priming kinase initiating phosphorylation of OMA-1 by GSK3 and subsequent recognition by a CUL2-based E3 ligase.[19,20] MEI-1 is the ortholog of mammalian katanin and is required in meiotic spindle organization but must be inactivated prior to mitosis. Phosphorylation by MBK-2 initiates the degradation of MEI-1 via APC-dependent ubiquitination.[22]

Roles of DYRK1A and DYRK1B in the Regulation of Protein Stability

The first results pointing to a role of DYRKs in cell cycle control via regulation of protein stability have been obtained in pioneering studies of DYRK1B.[23,24] DYRK1B destabilizes cyclin D1 by phosphorylating a threonine residue close to the C terminus.[23,25] A recent report suggests that DYRK1A can also catalyze this phosphorylation, leading to nuclear export and proteasomal degradation of cyclin D1.[26] The exact site of phosphorylation (Thr286 or Thr288) is controversial,

Table 1. Evidence for a role of DYRKs as regulators of protein stability

Kinase	Protein (phosphorylation sites)	GSK3 priming	Function	Comments	Ref.
Protein degradation					
DYRK2	c-Jun (S239)	yes	regulation of S-phase entry	DYRK2 /GSK3 initiate ubiquitination via Fbw7 E3 ligase	12
DYRK2	c-Myc (S62)	yes	regulation of S-phase entry	DYRK2 /GSK3 initiate ubiquitination via Fbw7 E3 ligase	12
DYRK2	GLI2 (S385, S1011)		effector of hedgehog pathway	DYRK2 reduces GLI2 levels, MG132 inhibitable	17
DYRK2	katanin p60 (S42, S109, T133)		control of mitotic transition	DYRK2 serves as a scaffold for EDVP E3 ligase	14
MBK-2	MEI-1 (katanin)		oocyte-to-embryo transition	MBK-2 initiates APC dependent degradation	21,22
MBK-2	OMA-1 (T239), OMA-2	yes	oocyte-to-embryo transition	MBK-2/GSK3 initiate ubiquitination by CUL2-based E3 ligase	19,20
DYRK1B DYRK1A	cyclin D1 (T286 or T288)	no	regulation of S-phase entry	phosphorylation initiates $SCF^{Fbx4/\alpha B\text{-}crystallin}$-mediated degradation	23, 26
DYRK1A	REST		neuronal differentiation	no direct evidence for phospho-degron; degraded via $SCF^{\beta\text{-}TrCP}$	35
DYRK1A	CRY2 (S557)	yes	component of circadian clock	SCF^{Fbxl3}-independent, MG132 sensitive	54
HIPK2	CtBP (S422)		transcriptional co-repressor	HIPK2 is required for the UV-induced decrease in CtBP	55
HIPK2	ZBTB4 (T783, T795, T797)		regulator of p21 expression	HIPK2 is required for the UV-induced decrease in ZBTB4	56
HIPK2	ΔNp63 (T397)		prosurvival factor	HIPK2 is required for DNA damage-induced degradation of ΔNp63	57
HIPK2	β catenin (S33,S37)	no	effector of Wnt pathway	$SCF^{\beta\text{-}TrCP}$-mediated degradation	58
HIPK2	Siah2 (S26,S28,S36)	no	E3 ligase involved in hypoxic regulation	phosphorylation reduces the half-life of Siah2	59
Yak1	cyclin B2		regulatory subunit of CDK	genetic evidence for enhanced APC-dependent degradation of cyclin B2	50
Protein stabilization					
DYRK1B HIPK2	$p27^{Kip1}$ (S10)		CDK inhibitor	phosphorylation enhances stability of p27 (by preventing nuclear export)	24,47
DYRK1A	HPV16E7 (Thr5, Thr7)		Viral oncoprotein	phosphorylation enhances stability	48
DYRK1A	Presenilin 1 (T354)		component of gamma secretase complex	phosphorylation enhances stability	60
DYRK1A	RCAN1 (T192)		inhibition of NFAT activation by calcineurin	phosphorylation enhances stability	61
Protein interaction					
DYRK1A/B HIPK2	DCAF7(= WDR68)		putative substrate receptor of CUL4-type E3 ligases	scaffold of HIPK2 complexes; DYRK1A recruits DCAF7 to the nucleus	40,41
DYRK1A	CUL9 (= PARC)		atypical E3 ligase	identified in interaction screen	43
DYRK1A	RNF216		E3 ligase	identified in interaction screen	43
DYRK2	DCAF1 (= VprBP)		substrate receptor of CUL4-type E3 ligases	identified by tandem affinity purification	14
Yak1	Hrt1 (= ROC1)		E2 recruiting subunit of SCF	identified in interaction screen	41

The table lists the DYRK substrates that are either destabilized or stabilized by phosphorylation and DYRK interacting proteins related to ubiquitin E3 ligases. The table includes proteins that are not related to cell cycle control as well as target proteins of HIPK2 that are not further discussed in the text

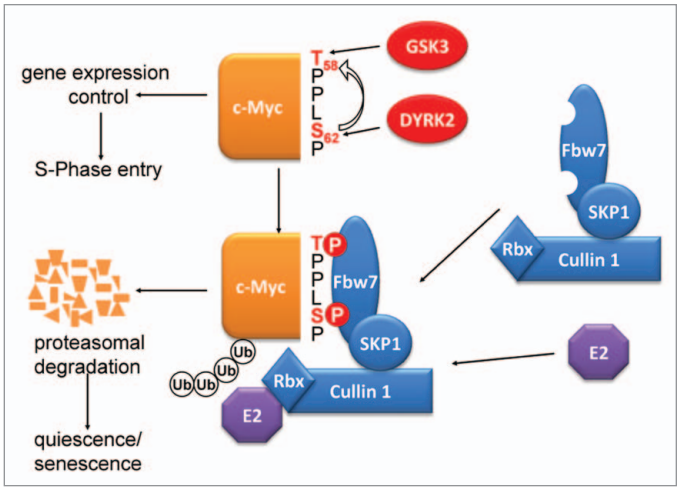

Figure 1. DYRK2 targets c-Myc for ubiquitination and destruction. Phosphorylation of Ser62 by DYRK2 primes c-Myc for phosphorylation at Thr58 by GSK3β. The resulting phosphodegron motif is recognized by Fbw7, which acts as the substrate receptor of an SCF complex (SKP1/cullin1/Fbw7/Rbx), initiating ubiquitination by the E2 ligase and subsequent proteasomal degradation. Likewise, c-Jun is ubiquitinated after sequential phosphorylation at Thr239 and Ser243 by DYRK2 and GSK3β.[12]

but it is clear that in this case, DYRK1A and DYRK1B do not act as priming kinases for GSK3. Phosphorylation on Thr286 by GSK3 in S phase is known to induce the cytoplasmic ubiquitination of cyclin D1 catalyzed by $SCF^{Fbx4/\alpha B\text{-crystallin}}$ E3 ligase.[27] DYRK1B rather appears to act in G_0/G_1 to maintain cells in growth arrest and quiescence by depleting cyclin D1.[22] In neurons, DYRK1A overexpression leads to the nuclear export and degradation of cyclin D1.[28] Importantly, cyclin D1 also plays a role in $p27^{Kip1}$ proteolysis, in the sense that loss of cyclin D1 causes accumulation of p27 (see below).[29] The importance of cyclin D1 proteolysis for normal cell homeostasis is highlighted by the fact that mutations in the cyclin D1 phosphodegron have been observed in human tumors.[30] It is worth mentioning that cyclin D2 and cyclin D3 are also phosphorylated on corresponding C-terminal threonines (Thr280 and Thr283, respectively) to trigger their UPS-dependent degradation.[31-33] It remains to be determined whether DYRK1A and/or DYRK1B also phosphorylate these cyclins.

The RE1-silencing transcription factor (REST) is expressed in dividing neural progenitors and acts as a repressor of neuronal differentiation and positive regulator of proliferation.[34] The neurodevelopmental effects of DYRK1A in Down syndrome may in part be due its effect on REST, since DYRK1A overexpression reduces REST protein levels through facilitating ubiquitination and subsequent degradation.[35] REST is regulated by phosphorylation and subsequent ubiquitin-mediated proteolysis in a $SCF^{\beta\text{-TRCP}}$ E3 ligase-dependent manner,[36] but it remains to be shown whether DYRK1A acts on this pathway. Reduced REST levels due to DYRK1A overexpression were documented from undifferentiated embryonic stem cells to adult brain and are predicted to favor cell cycle exit and differentiation of neural progenitor cells.[37]

Another strong indication that DYRK1A and DYRK1B are functionally linked with E3 ubiquitin ligases is the fact that both of them, as well as HIPK2 (but not DYRK2), have repeatedly been shown to interact with DDB1 and CUL4-associated factor 7 (DCAF7, also called WDR68 or Han11).[38-41] DCAFs are a family of more than 50 proteins that function as adaptor proteins of the CUL4-DDB1 ubiquitin ligases to mediate substrate specificity.[42] The specific function of DCAF7 as a receptor subunit of E3 ligase complexes is unknown, but one might speculate that it mediates the interaction either between the kinase and its substrate or between the kinase and an E3 ligase. Another protein interacting with DYRK1A is cullin 9,[43] which seems to be part of an atypical cullin-based E3 ligase complex and regulates p53.[44]

Stabilization of Target Proteins by DYRK1A and DYRK1B

In addition to targeting specific proteins for proteasomal degradation, DYRK1A or DYRK1B can stabilize other proteins by phosphorylation. The most pertinent example in this context is the phosphorylation by DYRK1B of $p27^{Kip1}$ on Ser10 during the G_0 phase of the cell cycle.[24] p27 is a CDK inhibitor that controls the transition from the G_1 into the S phase of the cell cycle. Phosphorylation on Ser10 stabilizes p27 in quiescent cells by maintaining the protein within the nucleus, where it inhibits CDK2.[45] The physiological importance of Ser10 phosphorylation was shown in lymphocytes from $p27^{S10A/S10A}$-knock-in mice, where protein turnover of p27 in G_0 phase, but not in S phase, was markedly enhanced compared with wild-type cells. Phosphorylation of Ser10 in G_1 phase or upon mitogenic stimulation is catalyzed by other kinases and has different functional consequences as compared with G_0.[46] Recently, HIPK2 has also been shown to phosphorylate Ser10 and stabilize p27 in asynchronously growing cell lines.[47] Further work will be necessary to uncover the contribution of the individual kinases in different cell types and different phases of the cell cycle.

The E7 oncoprotein of human papilloma virus type 16 (HPV16E7) is another substrate of DYRK1A and has been reported to be stabilized by phosphorylation on Thr5 and Thr7.[48] HPV16E7 induces the degradation of retinoblastoma family of proteins (pRb, p107 and p130) and promotes S phase entry. Phosphorylation by DYRK1A increased the half-life of HPV16E7 and enhanced the transforming potential of HPV16-infected cells. This effect is in striking contrast to the antiproliferative effects of DYRK1A or DYRK1B that result from phosphorylation of cyclin D1 or p27. Thus, the viral oncoprotein virtually hijacks and reprograms a cellular pathway that normally inhibits cell division.

Other Members of the DYRK Family

Yak1 is the first known DYRK and the only member of the family in *Saccharomyces cerevisiae*. The *Yak1* gene was identified in a genetic screen as a negative regulator of growth that is induced by arrest early in the cell cycle.[49] Furthermore, overexpression of *Yak1* suppressed defects in the degradation of cyclin B by the APC-ubiquitin-proteasome pathway, suggesting that the Yak1 kinase enhances the APC-mediated ubiquitination of cyclin B.[50] A large-scale interaction screen identified Yak1 as a protein binding to the Hrt1 component of the SCF E3 ligase complex.[51] Collectively, these results suggest that Yak1 may also be implicated in the regulation of protein turnover, but this point has not yet been addressed biochemically.

Conclusions and Perspectives

In conclusion, many pieces of evidence support the hypothesis that DYRK family kinases fulfill evolutionarily ancient functions in the regulation of protein turnover, either by the triggering UPS-mediated degradation or by enhancing the stability of target proteins. In line with the known roles of DYRKs in the regulation of cell proliferation and differentiation, many of the proteins whose abundance is modulated by DYRKs are involved in cell cycle control. Obviously, the effect on protein stability is not the only mechanisms by which DYRKs act as cell cycle regulators. For example, the proapoptotic effects of DYRK2 by phosphorylation of p53 and the role of DYRK1A in cell cycle exit by promoting assembly of the DREAM (DP, Retinoblastoma, E2F and MuvB) complex do not involve direct effects of DYRKs on protein turnover.[8,9,52] Moreover, DYRK1A upregulates $p27^{Kip1}$ levels not only by protein stabilization, but also by transcriptional regulation.[53]

It appears likely that more examples for a role of DYRKs as regulators of protein turnover will emerge, both in the regulation of the cell cycle and other cellular processes. Important tasks for the future include the elucidation not only of the exact mechanism of the interaction between DYRKs and the relevant E3 ligases, but also of the mechanism by which DYRKs can reduce the turnover of specific substrates (such as $p27^{Kip1}$).

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