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Chromatin-Binding Proteins Moonlight as Mitotic Microtubule Regulators

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Some microtubule (MT)-associated proteins bind to MTs and chromatin simultaneously to fulfill their mitotic spindle function. By contrast, a growing number of chromatin-binding proteins leave mitotic chromatin and interact with MTs via their chromatin-binding domains. I discuss this switch from chromatin to MT binding as a key regulatory principle of spindle formation.

Dynamic MTs Regulated by MT-Associated Proteins

MTs are highly-dynamic polymers of tubulin, important for multiple cellular processes. They are found exclusively in the cytoplasm during interphase and play essential roles in maintaining cell shape and intracellular transport. During mitosis, they rearrange into the bipolar spindle that drives chromosome segregation. To cope with these different roles, MT assembly and dynamics are precisely regulated in time and space by microtubule-associated proteins (MAPs). Their distinct functions are largely classified into: (i) *de novo* formation (nucleation) of MTs, (ii) subsequent stabilization or destabilization, and (iii) transport of cargos along the MTs (motor proteins) [1]. While some MAPs such as chTOG regulate MTs throughout the cell cycle, others reside in the nucleus during interphase, away from cytoplasmic MTs [1]. These nuclear MAPs become accessible to MTs when the nuclear envelope breaks down during mitosis, and play roles in spindle assembly and function.

In addition to nucleoplasmic proteins, some chromatin-binding proteins have been shown to regulate MTs during mitosis. Specifically, chromatin-associated MAPs bind to chromatin and MTs simultaneously via distinct chromatin- and MT-binding domains (Table 1). The chromokinesin kinesin-10/KID moves toward the plus-ends of MTs using its motor domain to promote chromosome alignment at the metaphase plate [2]. NuSAP binds to chromatin and locally stabilizes MTs, linking chromatin and MTs in the spindle [3]. By contrast, Dppa2 binds to chromatin and destabilizes MTs, and this destabilization is important for spindle disassembly in late mitosis [4]. While the functions of these MAPs during interphase remain unclear, recent work has shown that established chromatin regulators in interphase dissociate from chromatin in mitosis and regulate MTs as chromatin-dissociated MAPs.

Chromatin-Dissociated MAPs as New Temporal Regulators of MTs

Chromatin structure and function are dynamically regulated by the function of chromatin-binding proteins during the cell cycle and under particular cellular conditions. Transcription is induced from specific genes at specific timing, the genome is replicated during S phase, chromatin condenses in early mitosis and de-condenses later, and damaged DNA is repaired. The association of these chromatin-binding proteins is dynamic and temporally regulated. Many proteins dissociate from chromatin upon mitotic entry and bind again after mitosis, concomitantly with the structural rearrangement of chromatin into highly condensed mitotic chromosomes.

Chromatin-remodeling complexes modify chromatin by sliding, removing, inserting, or exchanging histones [5]. They bind to chromatin mostly via their catalytic ATPase subunit, but largely dissociate in mitosis [6]. These chromatin-remodeling ATPases are classified into four families (SWI/SNF, ISWI, CHD, and INO80), which share the SNF2-type ATPase domain but have distinct chromatin-binding domains [5]. Three of the ATPase families (Figure 1A) directly bind to MTs *in vitro* and localize to the mitotic spindle [6–9]. Depletion experiments in higher eukaryotes show that CHD4 and INO80 are required for spindle MT assembly [6,8], while ISWI is required for subsequent maintenance of the spindle during anaphase to promote chromosome segregation [7] (Table 1). Each ATPase has a different affinity for MTs. ISWI shows an approximate 10-fold higher affinity than CHD4, and can bind to the MT lattice with a molecular ratio of one ISWI per one tubulin dimer (Figure 1B) [6,7]. By contrast, SWI/SNF dissociates from mitotic chromatin, but its function in mitosis remains unknown [5].

The chromatin-bound nucleoporin MEL-28/ELYS, which plays a key role in post-mitotic nuclear pore complex (NPC) assembly [1], has been shown to bind to MTs *in vitro* [10]. In higher eukaryotes the NPC disassembles at mitotic onset concomitantly with nuclear envelope breakdown, and MEL-28/ELYS is completely removed from chromatin [10]. MEL-28/ELYS then relocalizes to the spindle poles and kinetochores in *Xenopus* egg extracts and human cells. There, MEL-28/ELYS promotes MT nucleation by recruiting the major MT nucleator, γ-tubulin [10]. Thus, many proteins have dual functions on chromatin in interphase and on the spindle in mitosis (Table 1).

Chromatin-Dissociated MAPs Bind to MTs via Chromatin-Binding Regions

Nuclear proteins are regulated by the Ran GTPase throughout the cell cycle [1]. They are translated in the cytoplasm and transported into the nucleus by interaction of their nuclear localization signal (NLS) with nuclear transport receptors. After import, the GTP-bound form of Ran (RanGTP), which is enriched in the nucleus, binds to transport receptors and liberates

Table 1. Chromatin-Associated MAPs and Newly Proposed Chromatin-Dissociated MAPs

Protein Name	Name Description	UniProt Entry (Human)	Proposed Molecular Function[a]	Binding Within Chromatin[b]	Refs
Chromatin-Associated MAPs					
Dppa2	Developmental pluripotency-associated protein 2	Q7Z7J5	M, MT depolymerization required for spindle disassembly in late mitosis	DNA	[4]
Kinesin-4/KIF4	Kinesin family member 4	O95239	M, chromokinesin required for chromatin condensation	DNA	[13]
Kinesin-10/KID	Kinesin-like DNA binding protein	Q14807	M, chromokinesin required for chromosome alignment to the metaphase plate	DNA	[2]
NuSAP	Nucleolar spindle-associated protein	Q9BXS6	M, linking MTs to chromosome arms	DNA	[3]
Chromatin-Dissociated MAPs					
CHD4	Chromodomain-helicase-DNA-binding protein 4	Q14839	I, chromatin-remodeling ATPase[c] M, MT stabilization	ND	[5,6]
INO80	Inositol requiring 80	Q9ULG1	I, chromatin-remodeling ATPase[c] M, required for MT assembly	ND	[5,8,9]
ISWI	Imitation switch/sucrose nonfermenting protein 2 homolog	O60264	I, chromatin-remodeling ATPase[c] M, MT stabilization in anaphase	Nucleosomes	[5,7]
KANSL1 KANSL3	KAT8-associated nonspecific lethal	Q7Z3B3 Q9P2N6	I, transcription M, MT minus-end stabilization	ND	[14]
MEL-28/ELYS	Maternal effect lethal-28/embryonic large molecule derived from yolk sac	Q8WYP5	I, NPC assembly M, MT nucleation	Nucleosomes	[1,10]
SSRP1	Structure-specific recognition protein 1	Q08945	I, transcriptional elongation M, required for spindle assembly	Nucleosomes	[15]

[a]I, interphase; M, mitosis.
[b]Information mainly from [11]. ND, not determined.
[c]Each chromatin-remodeling ATPase has been implicated in multiple chromatin regulations including transcription, DNA replication, and DNA repair. Therefore, their functions are not specified here.

nuclear proteins. This release exposes their NLS regions, which are often important for their localization and function. Chromatin-binding proteins frequently bind to chromatin via NLS-containing regions (Figure 1A), and MEL-28/ELYS, for example, binds to chromatin in a RanGTP-dependent manner [1]. RanGTP enrichment remains after nuclear envelope breakdown in mitosis, and forms a gradient around chromatin. Thus, RanGTP is able to release proteins from transport receptors and activate them near mitotic chromatin, including chromatin-dissociated MAPs for MT regulation.

Similar local activation in interphase and mitosis suggested that the NLS regions of the chromatin-dissociated MAPs participate in MT regulation. In contrast to chromatin-associated MAPs that have distinct chromatin and MT binding domains, domain analysis of the chromatin-dissociated MAPs revealed that ISWI, CHD4, INO80, and MEL-28/ELYS bind to MTs through regions containing chromatin-binding domains and NLSs (Figure 1A) [6,7,9,10]. Therefore, chromatin-binding domains and neighboring NLSs appear to be a minimal unit for RanGTP-dependent MT binding. While MT binding is crucial, other parts of the protein are also important for their function (Figure 1C). Indeed, full-length ISWI, but not the C-terminal MT-binding unit, is sufficient to rescue premature spindle disassembly during anaphase in ISWI-depleted *Xenopus* egg extracts [7].

While chromatin-associated MAPs bind to chromatin specifically in mitosis, chromatin-dissociated MAPs leave mitotic chromatin. The mechanisms for such chromatin association or dissociation are far from being understood. There is likely a common mechanism for the relocalization of many chromatin proteins rather than mechanisms unique to each protein. Proteomic analysis of chromatin-binding proteins in the presence or absence of nucleosomes, the fundamental unit of chromatin composed of histone octamers, revealed that chromatin-dissociated MAPs, including ISWI and MEL28/ELYS, are enriched on nucleosomes [11] (Table 1). *In vitro* experiments showed that MEL-28/ELYS directly binds to nucleosomes through an AT hook and the NLS in its C terminus [11] (Figure 1A,C), which is the same region required for MT binding [10]. By contrast, chromatin-associated MAPs such as kinesin 10/KID and Dppa2 are enriched on nucleosome-free