Actin in transcription

Actin is required for transcription by all three RNA polymerases in the eukaryotic cell nucleus

Actin is one of the most abundant proteins in eukaryotic cells. For many years, its presence in the nucleus has been considered an artefact, and the validity of the many reports published on nuclear actin has been questioned (reviewed by Pederson & Aebi, 2003). However, evidence has emerged in recent years supporting the direct functional involvement of actin, and of nuclear myosin 1 (NM1), in transcription. First, β -actin is associated with SWI/SNF-like chromatin-remodelling complexes of the BAF type in mammalian cells, and β -actin and other actin-related proteins have subsequently been identified as components of other chromatin-remodelling complexes (reviewed by Olave *et al*, 2002; Bettinger *et al*, 2004). Second, NM1 colocalizes with RNA polymerase II (Pol II), and antibodies against NM1- β co-immunoprecipitate Pol II and inhibit transcription *in vitro* (Pestic-Dragovich *et al*, 2000). Third, studies in a variety of organisms have revealed that β -actin interacts directly with some proteins from the heterogeneous nuclear ribonucleoprotein (hnRNP) complexes (Percipalle *et al*, 2002, and references therein). The association of actin with messenger-RNA-binding proteins is of functional importance, as has been shown by experiments using the dipteran *Chironomus tentans*. These studies have shown that disruption of the interaction between actin and the pre-mRNA-binding protein hrp65 by a competing peptide blocks Pol II transcription at the elongation level (Percipalle *et al*, 2003). An interaction between actin and human hnRNP U is also necessary for productive Pol II transcription (Kukalev *et al*, 2005). Finally, antibodies against β -actin inhibit Pol II transcription in a mammalian system (Hofmann *et al*, 2004). It is interesting that this inhibitory effect can be reproduced *in vitro* on naked DNA templates, which suggests that the role of actin in transcription is not mediated solely by chromatin remodelling. Hofmann and co-workers also showed that β -actin is a component of pre-initiation complexes and has an early role in transcription initiation (Hofmann *et al*, 2004).

There is thus no doubt that actin is involved directly in Pol II transcription, both in insects and mammals, and probably in all eukaryotes. Attention is now focused on the molecular mechanisms of actin function. Does actin have a role at the initiation level or at the elongation level, or both? Is actin required for chromatin remodelling or for the formation of pre-initiation complexes, or both? The different conclusions reached by different authors probably reflect a higher complexity of the role of actin in transcription than initially anticipated.

Indeed, three reports that were published during 2004 show that actin is also required for transcription by Pol I and Pol III (Fomproix & Percipalle, 2004; Philimonenko *et al*, 2004; Hu *et al*, 2004). Fomproix & Percipalle showed that the largest subunit of Pol I is associated physically with actin and NM1 in the nucleoli of human cells. They also showed in a series of *in situ* experiments that this association is disrupted when transcription is inhibited and, interestingly, that the nucleolar location of NM1 is sensitive to RNase digestion. This suggests that NM1 is bound to (pre)ribosomal (r)RNA. Grummt and co-workers have subsequently confirmed that actin and NM1 are associated with the Pol I machinery, and they have used biochemical methods to show that actin and NM1 are bound to a subpopulation of Pol I (Philimonenko *et al*, 2004). This subpopulation is able to support productive transcription and is characterized by the presence of the basal transcription initiation factor TIF-IA. The study also shows that Pol I activity is inhibited by anti-actin or anti-NM1 antibodies, both *in vivo* and *in vitro*, and that

Fig 1 | Actin and nuclear myosin 1 in transcription by RNA polymerase I. Actin is directly associated with RNA polymerase I (Pol I), regardless of whether Pol I is engaged in transcription, and nuclear myosin 1 (NM1) interacts with transcription initiation factor TIF-IA. Extracellular mitogens activate signalling cascades that lead to TIF-IA phosphorylation. Pol I is then recruited to the ribosomal DNA promoter through interaction with the phosphorylated TIF-IA, which brings actin and NM1 into close proximity with each other. Actin, but not NM1, remains associated with Pol I during transcription elongation.

overexpression of NM1 results in a dose-dependent increase of pre-rRNA synthesis. Both of these observations show that actin and NM1 are important in Pol I transcription. Interestingly, the anti-actin antibodies blocked Pol I, Pol II and Pol III activities to similar extents *in vitro*. Hu and colleagues also highlighted the requirement of actin for Pol III transcription. They showed that actin is associated with Pol III, is located at Pol III promoters *in vivo*, and is required for U6 transcription *in vitro* (Hu *et al*, 2004).

Perhaps the most novel issues raised by Philimonenko and colleagues (2004) are a possible physiological role for NM1 in the growth-dependent regulation of rRNA synthesis and the different functions of actin and NM1 in transcription. rRNA synthesis is regulated and responds to changes in the cellular environment, and TIF-IA has a central role in this regulation. TIF-IA is phosphorylated at serine (Ser) 649 and Ser 633 before it participates in the assembly of Pol I initiation complexes at the rDNA promoters. These phosphorylations are required for the subsequent interaction of TIF-IA with Pol I and with the TBP (TATA-binding protein)-containing complex (reviewed by Grummt, 2003). Philimonenko and co-workers have now shown that actin interacts directly with Pol I independently of transcription. NM1 binds to Pol I through TIF-IA, and the binding of NM1 to TIF-IA is independent of TIF-IA phosphorylation. Thus, actin and NM1 are only in close proximity during assembly of the initiation complex at the promoter (Fig 1). Furthermore, chromatin immunoprecipitation assays suggest that NM1 is restricted to promoter regions, whereas actin is associated with promoter regions and with transcribed sequences.

Many studies on nuclear actin and myosin assumed that these two proteins associate with each other, and that they probably act as molecular motors. Philimonenko and co-workers have shown clearly that actin and NM1 interact differently with specific components of the transcription machinery. It is clear that actin and myosin have specific, and to some extent independent, functions.

Research into the function of actin advanced significantly during 2004, and the view that has emerged is that actin is required by all three sets of transcription machinery in the eukaryotic cell nucleus. The challenge now is to understand the molecular mechanisms that underlie the many functions of actin in transcription, rather than question the functional significance of nuclear actin.

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