1 Introduction

1.1 RuvBL1 and RuvBL2 are highly conserved AAA$^+$ proteins

RuvBL1 and its homolog RuvBL2 are ubiquitously expressed proteins (Bauer et al. 1998) that belong to the AAA$^+$ family of ATPases (ATPases associated with diverse cellular activities) (Neuwald et al. 1999). This class of ATPases includes nucleic acid processing enzymes, chaperones and proteases. AAA$^+$ proteins often form hexameric ring structures and contain conserved motifs for ATP binding and hydrolysis like the Walker A (GXXXXGKT) and Walker B box (DEXH/N) (Walker et al. 1982), the Arg-finger and sensor residues. All AAA$^+$ proteins use ATP binding and hydrolysis to exert mechanical forces. ATP hydrolysis is clearly essential for the biological activity of RuvBL1 and RuvBL2 (Feng et al. 2003; Jonsson et al. 2004; Wood et al. 2000).

RuvBL1 and RuvBL2, consisting of 456 and 463 amino acids respectively, are mainly localised in the nucleus, but also found in the cytosol (Holzmann et al. 1998; Kim et al. 2006; Lim et al. 2000; Salzer et al. 1999). RuvBL2 exhibits 43 % sequence identity and 65 % sequence similarity to RuvBL1. These proteins were originally identified by several unrelated approaches and are therefore known under diverse names (see Table 1).

RuvBL1	RuvBL2	Explanation	Reference
TIP49	TIP48	**T**ATA-binding protein (TBP)-**i**nteracting **p**rotein	**(Makino et al. 1998; Wood et al. 2000)**
TIP49a	TIP49b	**T**BP-**i**nteracting **p**rotein	**(Kanemaki et al. 1999)**
Pontin52	Reptin52	**re**pressing **P**on**tin**52	**(Bauer et al. 2000; Bauer et al. 1998)**
TAP54α	TAP54β	**T**IP60-**a**ssociated **p**rotein	**(Ikura et al. 2000)**
TIH1	TIH2	**TI**P49a/b **h**omolog	**(Lim et al. 2000)**
ECP54	ECP51	**e**rythrocyte **c**ytosolic **p**rotein	**(Salzer et al. 1999)**
NMP238	---	**n**uclear **m**atrix **p**rotein	**(Holzmann et al. 1998)**
Rvb1	Rvb2	**RuvB** homolog	**(Jonsson et al. 2001)**
p50	p47	**p**rotein	**(Gohshi et al. 1999)**

Table 1: Different names of RuvBL1 and RuvBL2.

RuvBL1 and RuvBL2 share homology (~ 30 %) to the bacterial DNA-dependent ATPase and helicase RuvB (Putnam et al. 2001; Yamada et al. 2001), which is the motor that drives branch migration of the Holliday junction in the presence of RuvA and RuvC during homologous recombination and recombinational repair of damaged DNA (Tsaneva et al. 1993). The ruvA, ruvB and ruvC genes (Resistance UV) of bacteria are required for normal levels of cellular resistance to the effects of UV- or ionising-irradiation (Mezard et al. 1999). Helicases, like RuvB, are molecular motor proteins which couple the energy of ATP

hydrolysis to unwinding of the energetically stable duplex form of DNA or RNA and translocate along the nucleic acid in an ATP dependent manner (Figure 1). They play an essential role in many cellular processes which require single-stranded DNA or RNA devoid of secondary structures, such as DNA replication, transcription, DNA repair, recombination, RNA translation, splicing of RNA and assembly of ribosomes. Based on the limited sequence homology with RuvB it is speculated that RuvBL1 and RuvBL2 may be potential DNA translocation motors (Gohshi et al. 1999; Kanemaki et al. 1997; Makino et al. 1999). However, the original data reporting DNA helicase activities of RuvBL1 (Makino et al. 1999) and RuvBL2 (Kanemaki et al. 1999) were not reproducible with the purified wild-type proteins (Ikura et al. 2000; Qiu et al. 1998). It is therefore an open question whether RuvBL1 and RuvBL2 exert helicase activity.

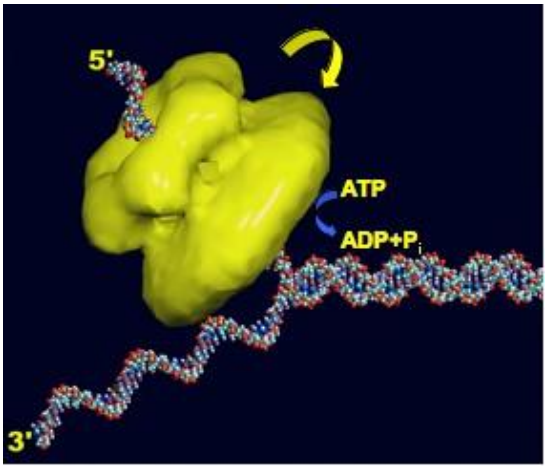

Figure 1: Working helicase. A hexameric 5´-3´ helicase from the bacteriophage T7 (yellow ring) couples the energy of ATP hydrolysis to unwinding of double-stranded DNA and translocates along the nucleic acid (www.msi.umn.edu; modified model).

The *RuvBL1* and *RuvBL2* genes are essential for viability in the yeast *Saccharomyces cerevisiae* (Qiu et al. 1998), in *Drosophila melanogaster* (Bauer et al. 2000) and in *Caenorhabditis elegans* (http://www.wormbase.org/). Their significant evolutionary conservation from yeast to man reveals that they mediate important cellular functions. Both proteins form a complex (RuvBL1/2) (Bauer et al. 2000; Puri et al. 2007) and work together in diverse cellular processes, but they also function independently from each other.

1.2 RuvBL1 and RuvBL2 are components of chromatin remodelling complexes

RuvBL1 and RuvBL2 were found to be involved in chromatin remodelling by several groups. A fundamental regulatory step in transcription and other DNA-dependent processes in eukaryotes is the control of chromatin structure, which regulates access of proteins to DNA. In the eukaryotic nucleus, DNA is wrapped around an octamer of four core histones in approximately two superhelical turns to form the nucleosome, and arrays of nucleosomes are successively folded into higher-order structures that collectively define chromatin (Figure 2). Packaging of genes into chromatin represses basal transcription and several multisubunit complexes are needed to regulate gene expression by modulating the topology of the nucleosomes in a number of ways. Two types of chromatin-modifying events that regulate chromatin structure and are important for DNA-based transactions in the cell have been identified (Figure 2).

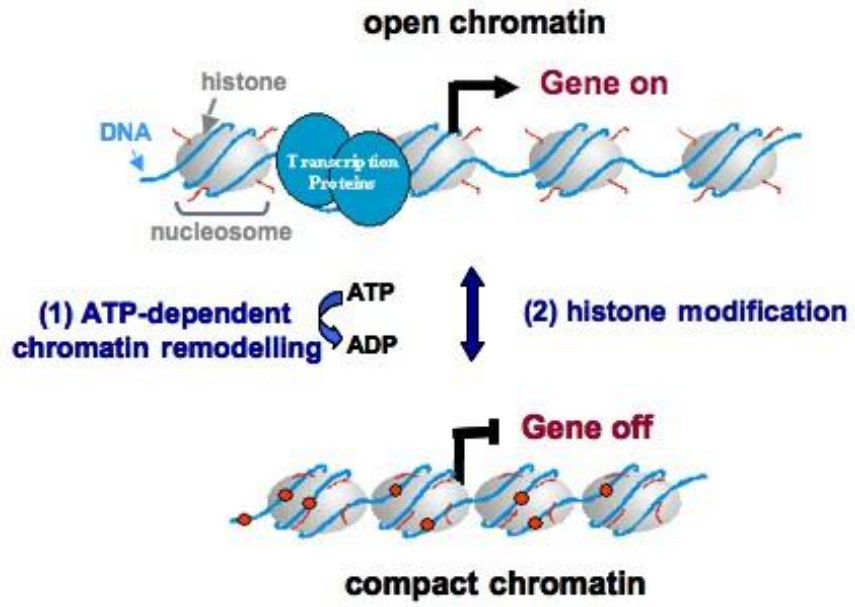

Figure 2: Regulation of chromatin structure. Packaging of genes into chromatin represses basal transcription. Two types of chromatin remodelling complexes regulate chromatin structure. (1) ATP-dependent complexes use the energy of ATP hydrolysis to modify nucleosome organisation and give access to the underlying DNA. (2) Histone modifiers add or remove covalent modifications onto histones to change the state of compaction of the chromatin or recruit histone-binding proteins.

The first is mediated by a group of ATP-dependent chromatin remodelling factors that use the energy of ATP hydrolysis to modify nucleosome organisation and give access to the underlying DNA. These complexes are classified according to their main ATPase. The four best-studied subfamilies are the SWI2 (switching), ISWI (imitation switch), CHD (chromo-helicase/ATPase DNA binding) and INO80 (inositol metabolism) subfamilies. The core

catalytic subunit of the INO80 complex contains a characteristic spacer in its ATPase domain and therefore belongs to the split ATPase subfamily which also includes SRCAP and p400. The SWI2, ISWI and CHD complexes are involved in nucleosome sliding and remodelling, while the split ATPase group is involved in nucleosome replacement. These histone-variant exchanger efficiently replace conventional histones with variant forms which are implicated in epigenetic control of transcription and in DNA repair processes (Jin et al. 2005a). The second type of chromatin remodelling factors is a group of enzymes that add or remove acetyl-, methyl- or phosphate-groups, ubiquitin or sumo modifications onto histones in the nucleosome core to change the state of compaction of the chromatin or recruit histone-binding proteins (Kouzarides 2007).

RuvBL1 and RuvBL2 were found to be present in the split ATPase group of chromatin remodelling complexes. They are present in two related complexes containing p400. The p400 complex is found in animal cells and is essential for E1A-mediated transformation and apoptosis (Fuchs et al. 2001; Samuelson et al. 2005). It is also involved in DNA repair (Kusch et al. 2004) and displays ATPase and helicase activities. It was shown that these functions are, at least in part, contributed by RuvBL1/2 (Fuchs et al. 2001). Interestingly, another p400-containing complex also contains the Tip60 histone acetyltransferase (HAT), and is therefore named the TIP60 complex. It performs critical functions in a variety of cellular processes including transcriptional activation, double strand DNA break repair and apoptosis (Ikura et al. 2000). RuvBL1/2 are also components of the yeast SWR1 complex and the corresponding SRCAP complex in animals (Jin et al. 2005b), which remodel chromatin by catalysing ATP-dependent replacement of H2A-H2B histone dimers in nucleosomes by dimers containing the histone variant Htz1 (referred to as H2AZ in mammalian cells) (Jin et al. 2005a; Mizuguchi et al. 2004). Finally, RuvBL1 and RuvBL2 are also found in the INO80 complex which exists in yeast and higher eukaryotes. It catalyses ATP-dependent sliding of nucleosomes along DNA and is involved in the repair of DNA double strand breaks and in transcriptional regulation (Jin et al. 2005b; Jonsson et al. 2001; Jonsson et al. 2004; Shen et al. 2000). It was shown that RuvBL1/2 are essential for the structural and functional integrity of the INO80 chromatin remodelling complex (Jonsson et al. 2004). RuvBL1/2 bound to ATP are in the correct conformation to associate with the INO80 complex and initiate the recruitment of the essential actin-like Arp5 subunit assembling the complete functional chromatin remodelling complex.

1.3 RuvBL1 and RuvBL2 are involved in transcription

RuvBL1 and RuvBL2 regulate transcription not only via association with chromatin remodelling complexes, but also through interactions with diverse transcription factors and the RNA polymerase II holoenzyme complex. First RuvBL1 and RuvBL2 were found to interact with the TATA-binding protein (Kanemaki et al. 1999; Kanemaki et al. 1997) and the large RNA polymerase II holoenzyme complex (Qiu et al. 1998), which contains over 50 components and is responsible for the transcription of protein-encoding genes. Later RuvBL1/2 were also identified by their physical interaction with the transcription-associated protein β-catenin (Bauer et al. 2000; Bauer et al. 1998) and with the transcription factors c-Myc (Wood et al. 2000), E2F1 (only RuvBL1 (Dugan et al. 2002)) and ATF2 (only RuvBL2 (Cho et al. 2001)). Since then, the mammalian homologs have been implicated in at least two oncogenic pathways, one involving c-Myc and the other one β-catenin. Among the transcription factors with oncogenic potential, c-Myc is one of the most frequent sites of mutation in human cancer (Cole 1986). The N-terminal portion of c-Myc contains two highly conserved regions, called Myc homology box I (MbI) and Myc homology box II (MbII). The MbII domain is necessary for virtually all c-Myc biological activities, including oncogenic transformation, apoptosis and the ability to block differentiation and stimulate cell proliferation (Evan et al. 1992; Li et al. 1994; Penn et al. 1990; Stone et al. 1987). This region was shown to bind to RuvBL1 and RuvBL2 (Wood et al. 2000). The Myc-binding site in RuvBL1 and RuvBL2 was mapped to amino acids 136-187 (Wood et al. 2000). A missense mutation in the RuvBL1 ATPase motif acts as a dominant inhibitor of c-Myc oncogenic activity but does not inhibit normal cell growth, indicating that functional RuvBL1 is an essential mediator of c-Myc oncogenic transformation (Dugan et al. 2002; Wood et al. 2000). RuvBL1 has also been shown to be a key modulator of apoptotic activity for both c-Myc and E2F1. Indeed RuvBL1 also binds to the E2F1 transactivation domain and modulates both transforming and apoptotic activities (Dugan et al. 2002). While no functional role has been established for RuvBL2 in these systems, the c-Myc N-terminus is proposed to recruit both RuvBL1 and RuvBL2 for transcriptional control of target genes critical for cell transformation.

RuvBL1 and RuvBL2 also functionally interact with β-catenin (Bauer et al. 2000; Bauer et al. 1998; Feng et al. 2003), which has a key role in Wnt signalling via effects on T-cell factor (TCF)-mediated transcription. The Wnt signalling pathway regulates many important processes, such as polarity of cell division, cell proliferation, and cell fate determination

(Bienz and Clevers 2000; Peifer and Polakis 2000). Mutational defects in β-catenin regulation are seen in many cancers, leading to elevated β-catenin levels, enhanced binding of β-catenin to TCFs, and increased expression of downstream genes, including *c-Myc* (He et al. 1998), *Cyclin D1* (Shtutman et al. 1999; Tetsu and McCormick 1999), *Cox-2* (Carlson et al. 2003) and *ITF-2* (Kolligs et al. 2002). RuvBL1 and RuvBL2 antagonistically regulate β-catenin signalling activity (Bauer et al. 2000; Feng et al. 2003). For example, Cyclooxygenase-2 (COX-2), a downstream target of the Wnt signalling pathway, is expressed early in colon carcinogenesis and is known to play a crucial role in the progress of the disease. RuvBL1 promotes COX-2 expression and is overexpressed in colon cancer tissue (Carlson et al. 2003; Lauscher et al. 2007). Expression of an ATPase-deficient mutant form of RuvBL1 (RuvBL1_D302N) inhibits β-catenin-mediated neoplastic transformation of immortalized rat epithelial cells and growth of human colon cancer cells with deregulated β-catenin (Feng et al. 2003). The RuvBL1_D302N mutant inhibits β-catenin-mediated activation of TCF-dependent cellular genes, which was linked to decreased acetylation of histones near β-catenin target gene sequences, suggesting that RuvBL1 mediates its transcriptional effects through local chromatin modifications (Feng et al. 2003). Acetylation of nucleosomal histones leads to relaxation of chromatin structure so that various transcription factors can gain access to and function on chromatin DNA (for reviews see (Schiltz and Nakatani 2000; Struhl 1998; Workman and Kingston 1998)). In contrast deacetylation of histones reverses this status. To control target genes, β-catenin may recruit chromatin modifying and remodelling cofactors.

While RuvBL1 can stimulate gene activation mediated by TCF and β-catenin, RuvBL2 represses gene activation (Bauer et al. 2000). Recent analysis of β-catenin targets in the mouse showed that RuvBL2 acts in complex with the co-repressor TLE1 (Groucho in *Drosophila*) and the histone deacetylases HDAC1 and 2, that localise to the promoters of the β-catenin targets at a time of development when their expression is silenced. Tissue culture experiments further showed that RuvBL2 and β-catenin are both required for repression. Furthermore the *liebeskummer* mutation (insertion of the amino acid residues FCR at the exon 7/8 junction) in the zebrafish homolog of RuvBL2 increases RuvBL2 ATPase activity and enhances its repressive effect on β-catenin-mediated transcription (Rottbauer et al. 2002). This opposite action of RuvBL1 and RuvBL2 on β-catenin-mediated transactivation constitutes a mechanism for the control of the Wingless/Wnt pathway.

There is an obvious parallel between the recruitment of RuvBL1 and RuvBL2 by c-Myc and by β-catenin oncogenic proteins. RuvBL1 acts as a positive effector in both cases, while RuvBL2 acts as a co-repressor that balances the co-activator function of RuvBL1 in the β-

catenin pathway. This functional relationship may have been conserved to provide a close control of transcriptional targets of several different pathways.

Another example for the repressing properties of RuvBL2 is given by its interaction with activating transcription factor 2 (ATF2) (Cho et al. 2001). ATF2 plays a central role in the control of the cell cycle and apoptosis in response to stress and DNA damage. Upon its phosphorylation on Ser-121, ATF2 associates with p300, which links it to the basal transcriptional complex (Kawasaki et al. 1998). Like p300, ATF2 was also reported to elicit histone acetyltransferase (HAT) activities. UV treatment or ATF2 phosphorylation increases its HAT as well as its transcriptional activities (Kawasaki et al. 2000). Furthermore ATF2 phosphorylation also increases RuvBL2-ATF2 association, which requires the C-terminal domain of RuvBL2 (Cho et al. 2001). RuvBL2 association with ATF2 attenuates ATF2 transcriptional activities and constitutes a novel mechanism to limit ATF2-mediated transcription. Whereas ATF2 emerges as an inhibitor of the cell cycle and induces apoptosis in response to DNA damage, both c-Myc and β-catenin potentiate growth, replication, and transformation. In contrast to c-Myc and β-catenin, which interact with both RuvBL1 and RuvBL2, ATF2 only binds to RuvBL2. RuvBL1 neither binds to ATF2 nor affects its transcriptional activities (Cho et al. 2001).

As mentioned above, in some cases RuvBL2 gets recruited to promoters, and brings along a histone deacetylase that deacetylases histones and thereby converts the chromatin to a more repressive state. The repressive potential of RuvBL2 may also be controlled by sumoylation, which enhances the interaction with the histone deacetylase (Kim et al. 2006). Interestingly, metastatic cells contain higher levels of the small ubiquitin-like modifier (SUMO)-conjugating enzyme Ubc9, which attaches SUMO to lysine 456 of RuvBL2, and lower levels of the SUMO-processing enzymes SENP1 and SUSP1, which bind to RuvBL2 and remove SUMO (Kim et al. 2006). As a consequence, a larger fraction of RuvBL2 is sumoylated in metastatic cells, and Kim *et al.* showed that sumoylation stimulated the repressive potential of RuvBL2 by increasing its interaction with the histone deacetylase HDAC1.

An additional way of modulating the transcriptional activity of RuvBL1 and RuvBL2 was revealed with the identification of the interacting protein Hint1 (histidine triad nucleotide-binding protein 1), which acts as a co-regulator in TCF-β-catenin-mediated transcription (Weiske and Huber 2005). Hint1 directly binds to RuvBL1 and RuvBL2 thereby affecting RuvBL1/RuvBL2 complex formation. The Hint1-binding region in RuvBL1 and RuvBL2 was localised to amino acid residues 214-295 and 218-289, respectively. Weiske and Huber

suggest that this region serves for multimerisation between both, because binding to Hint1 disrupts formation of RuvBL1/RuvBL1, RuvBL2/RuvBL2 and RuvBL1/RuvBL2 complexes, but does not interfere with the association of RuvBL1 or RuvBL2 with β-catenin. Thereby, Hint1 reduces the ability of RuvBL1 to activate reporter constructs or endogenous β-catenin targets such as *axin2* and *cyclin D2* (Weiske and Huber 2005). In general it is possible that RuvBL1 and RuvBL2 exert specialised functions through individual interactions with other proteins, which disturb RuvBL1/2 complex formation.

Another complex containing both RuvBL1 and RuvBL2 was purified from vertebrate cells and called Uri1 complex. It has a role in the cellular response to extracellular nutrient levels and interacts physically with RNA polymerase II. Uri1 is also important for the maintenance of genomic integrity, as loss of *uri1* results in an increased number of double strand breaks in the nematode *Caenorhabditis elegans* (Parusel et al. 2006). These different observations provide an additional link between transcription, the repair of double strand breaks and the activity of RuvBL1 and RuvBL2, although their exact roles within the Uri1 complex still need to be elucidated.

Using yeast as model organism, Jonsson and co-workers revealed that RuvBL mutants unable to bind and/or hydrolyse ATP show rapid changes in the transcription of over 5 % of genes, with a similar number of genes being repressed and activated (Jonsson et al. 2001). There is only a limited overlap between the genes regulated by RuvBL1 and RuvBL2 suggesting that RuvBL1 and RuvBL2 have non-redundant roles in the activation or repression of specific genes. Taken together RuvBL1 and RuvBL2 represent transcriptional cofactors that function in diverse pathways.

1.4 Additional roles of RuvBL1 and RuvBL2 in snoRNP assembly and mitosis

Small nucleolar ribonucleoprotein (snoRNP) particles are complexes consisting of small nucleolar RNAs (snoRNAs) and associated proteins. These complexes are displaced to the nucleolus after synthesis in the nucleoplasm. SnoRNPs function in the modification and processing of pre-ribosomal RNA and are therefore essential for ribosome biogenesis (Filipowicz and Pogacic 2002). RuvBL1 and RuvBL2 are involved in snoRNP assembly and maturation in the nucleoplasm (King et al. 2001; Watkins et al. 2004). It was shown that RuvBL2 is exclusively associated with the precursor complex (Watkins et al. 2004), while

RuvBL1 is still associated with the maturing snoRNP complex. The interaction with RuvBL2 might represent transient association during early stages of biogenesis, coupling snoRNA synthesis with snoRNP assembly and localisation. It was proposed that RuvBL1 is a protein remodelling factor that mediates structural changes during final maturing events in the nucleoplasm. However, RuvBL1 is no longer bound to the mature nucleolar complex. These findings suggest that RuvBL1 and RuvBL2 may directly interact with RNA, but this assumption still has to be verified.

The repertoire of RuvBL1 and RuvBL2 functions was further extended by observations that both are associated with centrosomes and elements of the mitotic apparatus in mitosis (Gartner et al. 2003; Sigala et al. 2005). In case of RuvBL1 it was shown that these associations occur via interactions with tubulin (Gartner et al. 2003). Interestingly RuvBL1 and RuvBL2 show distinct localisations during telophase and cytokinesis (Sigala et al. 2005). These findings suggest that RuvBL1 and RuvBL2 may have separate individual functions in mitosis which are distinct from their role in transcription and chromatin remodelling. Taken together RuvBL1 and RuvBL2 are involved in many important cellular processes (Figure 3). However, the mechanism mediating their function is still unclear.

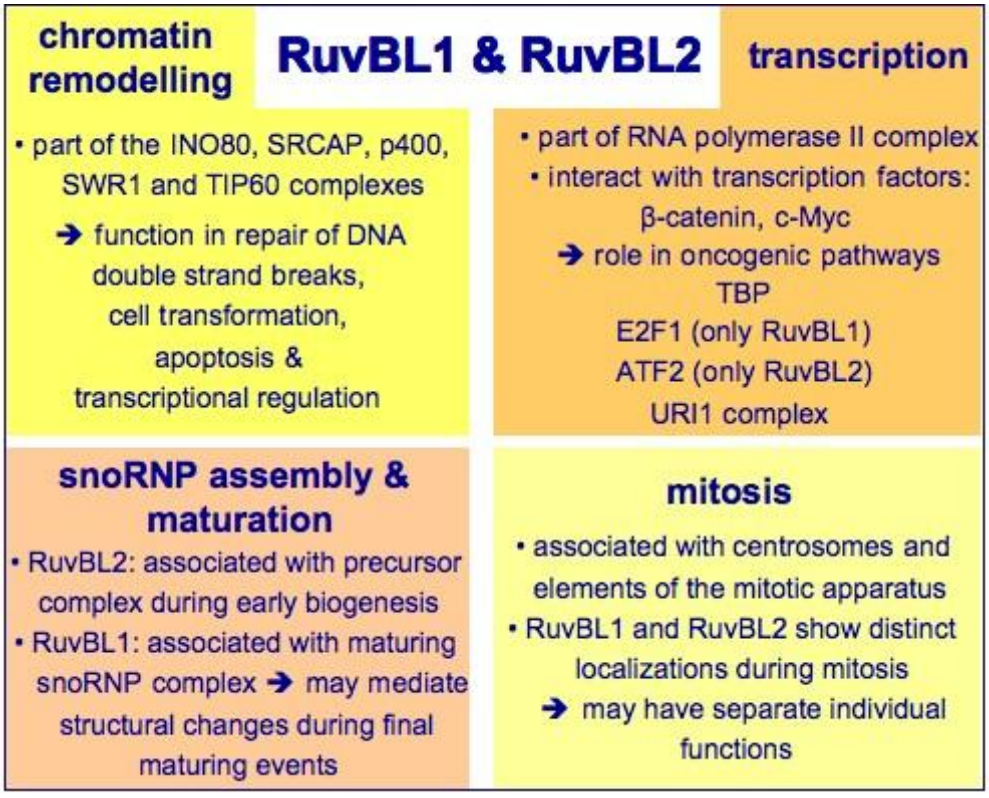

Figure 3: RuvBL1 and RuvBL2 are involved in diverse cellular processes. Both proteins play important roles in chromatin remodelling, transcription, snoRNP synthesis and mitosis. They have been implicated in at least two oncogenic pathways via direct interactions with c-Myc and β-catenin.