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Review Article

Toll-Dorsal Pathway Regulates Immunity as well as Dorso-Ventral Patterning in *Drosophila*

MANISH CHAUHAN, PRASHANT PRADHAN and NIROTPAL MRINAL*
Laboratory of Molecular Biology, South Asian University, Chanakyapuri, New Delhi 110 021, India

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The identification of the Toll receptor in *Drosophila* was a major discovery which restructured the paradigm in immunity as well as developmental biology. Toll homologues are present in mammals where they regulate only immunity. However, in flies, same Toll pathway controls two disparate biological processes like embryonic dorso-ventral patterning and humoral immune response. Toll activation by Spätzle is preceded by a proteolytic cascade which is differently regulated in immunity and embryonic development. Signalling downstream to Toll is similar in both processes and eventually leads to the activation of *Drosophila* homologues of NF-κB proteins; Dorsal and DIF. Toll pathway, in the early embryo results in graded expression of Dorsal protein leading to Dorso-Ventral axis patterning. On the other hand, activation of Toll-Dorsal pathway in the fat-body leads to induction of antibacterial proteins in larval and adult stages. Here, we review the current status of our knowledge of Dorsal pathway in embryonic development and immunity with special emphasis to regulation of target gene/s of the two pathways by Dorsal.

Key Words : Dorsal; Toll; DNA Geometry; Dorso-Ventral Patterning; Insect Immunity

Introduction

Drosophila has been used to dissect genetic basis of different phenotypes and pathways which has helped us in understanding many biological paradigms (Ashburner and Carson, 1986; Fortini *et al*., 2000). One such paradigm was the discovery of primary role of Toll receptors in embryonic development (Nusslein-Volhard and Wieschaus, 1980; Stein *et al*., 1991). Subsequent study led to the finding of rest of the downstream genes of dorsal-ventral (D-V) patterning of the embryo. These genes are collectively called as 'the dorsal group of genes' which includes *Toll, tube, pelle, cactus,* the *NFκB* homolog *dorsal*. In the year 1996, it was conclusively shown that 'dorsal group of genes' from the Toll ligand *spätzle (Spz)* to *cactus,* is involved in the anti-fungal response in *Drosophila* (Lemaitre *et al*., 1996). This set a platform for the discovery and characterization of *Toll*-like receptors in humans (Medzhitov *et al*., 1997) establishing the role of the *Drosophila* Toll-pathway as an evolutionarily conserved mechanism. However, mammalian TLRs are believed to have no role in development (Kimbrell and Beutler, 1998), whereas the *Drosophila* Toll-pathway is involved both in immunity and developmental processes (Halfon *et al*., 1995; Lemaitre *et al*., 1996). Here, we try to understand, how *Drosophila* Toll-pathway controls both embryonic patterning and immune response.

Toll receptor of Drosophila

The *Toll* gene was discovered as a component of D-V patterning pathway in *Drosophila* early embryos (Anderson and Nüsslein-Volhard, 1984; Anderson *et al*., 1985). *Drosophila Toll* belongs to a family of

*Author for Correspondence: E-mail: nmrinal@sau.ac.in; Tel.: 011-24195227

nine paralogues. These are *Toll*, *18 wheeler* (*18w*), *Toll-3* (also called *MstProx*), *Toll-4*, *Toll-5* (also called *Tehao*), *Toll-6*, *Toll-7*, *Toll-8* (also called *Tollo*), and *Toll-9*. Nearly all of the *Drosophila* paralogues share the same protein architecture. The main difference between the *Toll* paralogs is in the number of LRRs. The exception is *Toll-9*, which has a single block of LRRs in the ectodomain, compared to the two blocks seen in the other Tolls (Imler and Hoffmann, 2001). *Toll-9* is more closely related to the vertebrate Toll-like receptors (TLRs) than other *Drosophila* Tolls. Of all Toll proteins of *Drosophila*, Toll-1 is well studied and is the regulator of D-V patterning and immune response.

Toll expresses maternally in early embryos, and zygotically during late embryonic development. Zygotic Toll is expressed in the gut, salivary glands and epidermis (Gerttula *et al*., 1988). Toll is expressed in migrating cells, and also localises to the sites of contact between the fused epidermal layers at the dorsal midline (Hashimoto *et al*., 1991). *Drosophila* Toll receptor is a type I transmembrane protein. It comprises of an N-terminal ecto-domain, middle transmembrane domain, and the C-terminal intracellular domain. The ecto-domain is involved in interaction with other proteins through leucine-rich repeats (LRRs). The LRR domain in Toll is interrupted by two Cysteine rich domains (CRD), thus dividing the LRRs into two blocks. Second CRD is sandwiched between the C-terminal LRR and the transmembrane domain. The intracellular region of Toll contains a Toll/Interleukin-1 Receptor (TIR) domain, which initiates intracellular signalling upon recruitment of adapter proteins.

Toll in Dorso-Ventral Patterning

Bilateral symmetry in animals results from the presence of the Anterior-Posterior (A/P), and, Dorso-Ventral (D/V) axis. In *Drosophila*, D-V patterning pathway gets activated in the maternal egg chamber itself. This pathway is triggered upon somatic expression of Nudel, Pipe and Windbeutel proteins which results in the establishment of ventral cell fates (Stein *et al*., 1991; Dissing *et al*., 2001). Pipe is expressed on the ventral side of the follicular epithelium, while Nudel, a protease, is uniformly expressed, (LeMosy *et al*., 1998; Zhu *et al*., 2005). Next step is activation of proteolytic cascade involving three proteases, Gastrulation defective (Gd), Snake and Easter, in the perivitelline space (Han *et al*., 2000; Dissing *et al*., 2001; Cho *et al*., 2010). The final proteolytic step releases mature Spz from its inactive pro-peptide form by Easter, after which it binds Toll (Stein *et al*., 1991; Schneider *et al*., 1994; DeLotto and DeLotto, 1998; Weber *et al*., 2003). Cleaved Spz is a dimer, and binding of one Spz dimer to Toll induces receptor homo-dimerization (Gangloff *et al*., 2008). Recently it has been shown that Toll dimerization is accompanied by endocytosis of this complex (Huang *et al*., 2010).

On the inner side of the cell membrane, the intracellular TIR domain of Toll interacts with proteins containing Death-domain, which are collectively called as adapter proteins. The three adaptor proteins of Toll pathway are MyD88, Tube and Pelle (Hecht and Anderson 1993; Shen *et al*., 2001; Kambris *et al*., 2003). Upon binding of Spz, the TIR-domain of Toll protein forms a homotypic interaction with the TIR domain of MyD88, resulting in the recruitment and activation of the other two adapter proteins (Kambris *et al*., 2003). Subsequent step in the Toll signalling is the activation of transcription factor, Dorsal. In the cytoplasm Dorsal remains bound to its inhibitor protein called Cactus (Whalen and Steward, 1993). Upon Toll activation, Cactus gets phosphorylated ultimately leading to its degradation by the ubiquitin-proteasome pathway (Bergmann *et al*., 1996, Reach *et al*., 1996). In the process, Dorsal gets released and this free Dorsal enters the nucleus to induce or repress target genes.

Regulation of target gene expression by Dorsal is concentration dependent. On the ventral side, concentration of Dorsal is maximum which leads to activation of genes like *twist* and *snail*, which specify the mesoderm. On the lateral surface, Dorsal expression tapers down and in this region, it activates *sim* which specifies meso-ectoderm and *rho* and *sog* which specify neuro-ectoderm (Stathopoulos *et al*., 2002; Stalhopoulos *et al*., 2004; Biemar *et al*., 2006). On the dorsal surface, there is no expression of Dorsal

at all, and here *zen* is transcribed. Transcription of *zen* on the ventral and lateral surface is inhibited by Dorsal (Huang *et al*., 1997; Stathopoulos and Levine, 2002). The induction and repression of target genes by Dorsal in a concentration dependent manner is regulated by affinity of Dorsal to its binding sequence (*κB* motif) (Biemar *et al*., 2006). It has been proposed that genes like *twist* and *snail* which are expressed on the ventral surface (where Dorsal expression is highest), possess low affinity *κB* motif, while genes like *dpp* and *zen* which are expressed on the dorso-lateral surface (where Dorsal expression is low) have high affinity *κB* motifs (Ip *et al*., 1991; Jiang *et al*., 1992; Kirov *et al*., 1994; Dubnicoff *et al*., 1997; Huang *et al*., 1997; Stathopoulos *et al*., 2004; Hong *et al*., 2008). This is how the gradient of nuclear Dorsal, triggered by ventrally restricted Toll signalling, establishes the dorso-ventral axis in *Drosophila* embryo.

Toll in Innate Immunity

Drosophila was shown to have an inducible antibacterial system way back in 1970s (Bakula, 1970; Boman *et al*., 1972; Boman *et al*., 1974). Since *Drosophila* does not have an adaptive immune arm hence this offers the cleanest system to explore humoral response (Tzou *et al*., 2002). Humoral immune response in *Drosophila* is mediated by Anti-Microbial Proteins (AMPs) which are produced in the fat body (analogous to mammalian liver) and secreted into the hemocoel (Boman *et al*., 1974; Lemaitre *et al*., 1996; Carton *et al*., 1997; Lemaitre *et al*., 1997; Basset *et al*., 2000; Imler and Bullet, 2005). Post bacterial challenge AMP response persists for several days and gives protection against microbes. *Drosophila* secretes a total of 55 AMPs upon infection by diverse pathogens, which are grouped into seven major classes based on amino acid compositions (Basset *et al*., 2000; Hultmark, 2003). Prophenoloxidase activation precedes AMP activation and is very critical for insect immunity (Soderhall and Cerenius, 1998). These AMPs are not microbe specific e.g. drosomycin and metchniknowin are active against fungi; attacin, cecropin, diptericin and drosocin are active against Gram-negative bacteria while defensins are active against Gram-positive bacteria (Boman *et al*., 1974; Lemaitre *et al*., 1996; Lemaitre *et al*., 1997; Levashina *et al*., 1999; Hoffmann, 2003; Brennan and Anderson, 2004; Vierstraete *et al*., 2004). Activation of these genes requires triggering of the Toll or IMD pathway. Flies deficient for both the Imd and Toll pathways fail to induce any of the known antimicrobial peptides and succumb readily to infection (Lemaitre *et al*., 1996; Tzou *et al*., 2002; Hoffmann, 2003; Hultmark, 2003; Brennan and Anderson, 2004). Moreover, activation of either pathway, for example; by over-expressing a pathway component, is sufficient to trigger AMP expression in the absence of infection (Levashina *et al*., 1999; Georgel *et al*., 2001; Imler *et al*., 2004). In this review we will restrict to Toll pathway only.

Extracellular Toll Signalling

Pathogens on their surfaces express molecules called as Pathogen-Associated-Molecular-Patterns (PAMPs), which are recognized by the host for mounting immune response (Irving *et al*., 2001). The molecular system in the host that recognizes these PAMPs are called as Pathogen Recognition Receptors or PRRs. *Drosophila* has two families of PRRs: Peptidoglycan (PGN) recognition proteins (PGRP) and Gram-negative binding proteins (GNBP), which identify distinct PAMPs associated with Gram-positive (LYS-type PGN); Gram-negative bacteria (DAP-type PGN); or fungi (Beta-1,3-glucan) (Gottar *et al*., 2006; Ferrandon *et al*., 2007). Circulating GNBP3 in hemolymph directly recognizes Beta-1, 3-glucan of fungi and triggers downstream signalling, that finally activates the Toll pathway (Buchon *et al*., 2009; Levashina *et al*., 1999). In contrast, the PGN of Gram positive bacteria are recognised by PGRP-SA/PGRP-SD and GNBP1. Formation of PAMP and PRR complex activates ModSP (modular of serine proteases) which, in turn, triggers a proteolytic cascade that results in the activation of Spz, the ligand of Toll (Kambris *et al*., 2006). However, the proteolytic pathway that gets activated in immunity is different from the one that is used in dorso-ventral patterning and this is how the two processes distinctly regulate Toll activation (Fig. 1). The first serine protease in the immune cascade is ModSP, and recognizes both bacterial and fungal PAMPs (Buchon

et al., 2009). Parallel to ModSP is the protease Persephone (Levashina *et al*., 1999; Ligoxygakis *et al*., 2002). Interestingly, Persephone can activate Toll independently of PRRs and is said to respond to 'danger signals' associated with infection (El Chamy, 2006; Kambris *et al*., 2006). Next protein in the pathway is Sphieroide, a serine protease, which further regulates Spatzle Processing Enzyme (SPE). SPE directly cleaves Spz, releasing the Cys-knot in the Spz, which is critical for binding Toll (Jang *et al*., 2006).

Intracellular Toll Signalling

Unlike the extracellular proteolytic cascade, the intracellular signalling cascade activated by Toll during immune response is similar to that in the D-V patterning (Gay and Gangloff, 2007). In spite of the similarity in the intracellular signaling, the effector molecule induced in immunity is not Dorsal but its homologue named DIF (Dorsal related Immunity Factor) (Manfruelli *et al*., 1999; Meng *et al*., 1999). In the larva, DIF and Dorsal act redundantly, but in the adult, DIF is alone required for mounting immune response and not Dorsal (Rutschmann *et al*., 2000). The target genes of the Toll-Dorsal signalling pathway during immunity include anti-microbial peptides like drosomycin (Lemaitre *et al*., 1996).

Toll has an intracellular TIR domain, which recruits adapter proteins, MyD88, Tube and Pelle, to the Spz bound activated Toll receptor (Fig. 1). All three adaptor proteins contain a Death-domain, which is involved in protein-protein interactions leading to formation of a trimer complex (Hecht and Anderson, 1993; Kambris *et al*., 2003). MyD88 and Tube dimer is localised to the plasma membrane. The Death-domain of Pelle then binds that of Tube (Sun *et al*., 2002). Pelle also carries a kinase domain and triggers the activation of Dorsal, possibly through interaction with dTRAF2 (Shen *et al*., 2001). Interestingly, dTRAF2 is required for Toll signalling in immunity, but is not essential for embryogenesis: as *dTRAF2* null mutant flies survive to adulthood (Cha *et al*., 2003).

In the absence of signalling, Dorsal is found in the cytoplasm in complex with IκB homologue

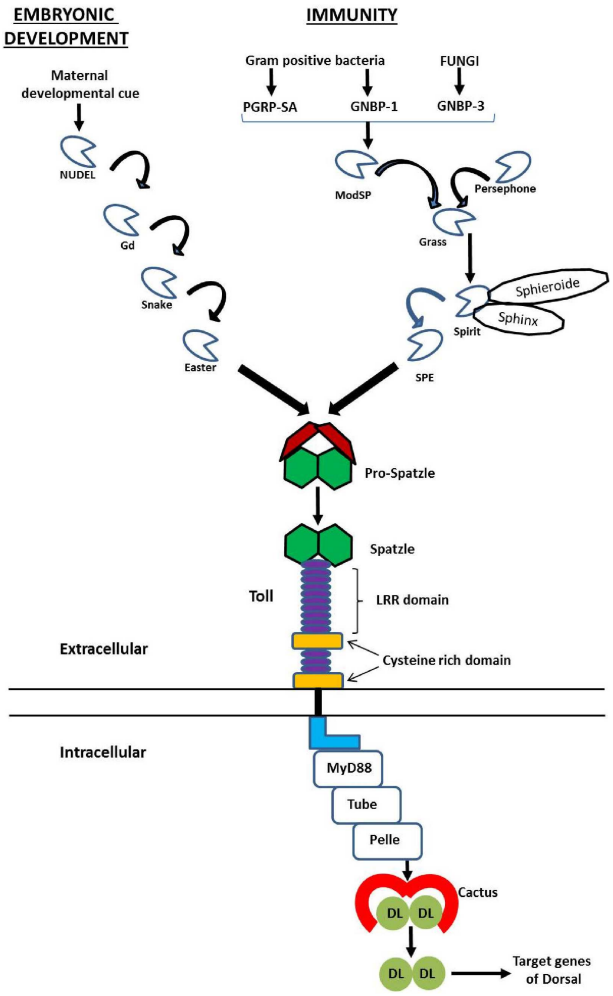

Fig. 1: The figure shows the activation of Toll pathway in embryonic development and immunity. Note that the difference is only in the signalling upstream to activation of Spz which is the ligand for Toll. During development Spz activation is through proteases Nudel, Gd, Snake and easter to SPE (Spz processing enzyme). While during immune response the Spz processing is through ModSP, Grass, Sphieroide pathway which activates SPE leading to processing of Spz. Signalling to SPE is through different PRRs (GNBP, PGRPs) which recognise different PAMPs (Beta 1,3-glucan, Lys type PGN etc). Activated Spz binds Toll receptor inducing structural changes which in turn activates the cytoplasmic TIR domain resulting in interaction with Death domain proteins MyD88, Tube and a kinase named Pelle. This is followed by phosphorylation of Cactus which in turn marks it for proteolytic degradation. This results in release of Dorsal/ DIF which enter the nucleus to regulate gene transcription

Cactus (Whalen and Steward, 1993). Activation of Toll signalling leads to the degradation of Cactus and the nuclear localisation of Dorsal to direct gene expression (Bergmann *et al*., 1996). The phosphorylation of both Dorsal and Cactus is required

for the nuclear localisation of Dorsal (Gillespie and Wasserman, 1994; Drier *et al.*, 1999). Phosphorylated Dorsal interacts with dNTF2 (Drosophila Nuclear Translocation Factor 2) and Nup88 to localise to the nucleus.

Once inside the nucleus, Dorsal/DIF rapidly activates AMPs, which are later released in the hemolymph (Hoffmann, 2003). AMPs are small, secreted, cationic peptides that can kill microbes or block their growth by disrupting membrane integrity (Hoffmann, 2003; Tanzi *et al*., 2010). AMP gene loci regulated by the Toll pathway have a single *κB* site characterized by a GGGAA consensus half site. While the regulation of target AMP genes by Dorsal/DIF upon microbial infection is well established, transcriptional regulation of Dorsal or DIF is not known. We found that in dl^1 mutant, expression of Dorsal protein was not detected (Mrinal and Nagaraju, 2010). This indicated towards a possible auto-regulation of *dl* gene by Dorsal itself. Recently, we have explained the mechanism of auto-regulation for Dorsal (Mrinal and Nagaraju, 2010). We found that early in immune response, Dorsal induces transcription of its own gene by binding to a *κB* motif present in the first intron of *dl* gene. However, in the later stages of immune response, Dorsal repositions itself to another binding site in the promoter of *dl* gene (Mrinal and Nagaraju, 2010). Interestingly, Dorsal binding to the promoter *κB* motif results in transcriptional repression of *dl*. We have shown that the repressor function of Dorsal is due to co-operative interactions with a co-regulator AP-1 (*Drosophila* homologue) protein. This established the temporally regulated feedback loop in Dorsal auto-regulation (Mrinal and Nagaraju, 2010). Auto-activation and auto-repression of genes has been known for a long time. However, Dorsal auto-regulation is the first example of its kind, where both auto-activation and auto-repression of a gene is affected by the same transcription factor. We were puzzled to notice that the two *κB* motifs in the *dl* gene bind the same transcription factor and still produce opposite outcomes. This prompted us to further explore the mechanism of *dl* auto-regulation by Dorsal protein.

Mechanism of Gene Activation and Repression by Dorsal

Dorsal is a transcription factor belonging to NF*κ*B family of proteins. TFs of this family can act both as activator and/or repressor of transcription. In *Drosophila*, Torso receptor tyrosine kinase signalling pathway, which is activated at the poles, selectively masks the ability of Dorsal to function as a transcriptional repressor at these positions (Rusch and Levine, 1994; Rusch and Levine 1996). Torso signalling modulates the ability of Capicua (Cic), Cut, and Dri, proteins that consequently influence the ability of Dorsal to function as a transcriptional repressor (Valentine *et al*., 1998; Jimenez *et al*., 2000). This suggests that activation or repression by Dorsal is dependent on a potential crosstalk with other pathways.

First, classical study based on the analysis of cis-regulatory sequences showed that Dorsal can function either as an activator or a repressor to control gene expression along the D-V axis (Jiang *et al*., 1993, Kirov *et al*., 1994). Dorsal, by default, is an activator of transcription in *Drosophila* and its repressor function is context-dependent, *e.g.*, repression of *zen* by Dorsal was ascribed to cooperative interactions with DNA-binding proteins occupying associated AT-rich sequences upstream of Dorsal binding site in the *zen* promoter (Kirov *et al*., 1993). It was also shown that repressor activity of Dorsal requires Groucho, a global co-repressor (Dubnicoff *et al*., 1997). We have already shown that, repressor activity of Dorsal was co-repressor dependent (Mrinal and Nagaraju, 2010). Both these studies have suggested that repressor function of Dorsal is co-regulator dependent. However, it has raised another query as to why Dorsal interaction with co-regulator is possible at few *κB* motifs but not on others?

Role of Combinatorial Code in Target Gene Regulation by Dorsal

Combinatorial interactions between Dorsal and other transcription factors were found to be important in spatio-temporal regulation of target gene expression. Synergistic DNA binding between Dorsal and Twist permits gene expression in more lateral regions of

the embryo where, neither Dorsal nor Twist is capable of inducing gene expression alone (Gonzalez-Crespo and Levine, 1993 and 1994; Jiang *et al*., 1993; Stathopoulos and Levine, 2002). Furthermore, transcriptional repressors function to refine the expression domains produced by activators. For instance, to restrict *rho* expression to ventro-lateral stripes in the embryo, Dorsal and Twist activation is antagonized in ventral regions by the Snail repressor, resulting in the lateral stripe pattern of gene expression exhibited by these genes (Kosman *et al*., 1991; Ip *et al*., 1992). Advent of genomics has allowed scientists to dig further this concept of combinatorial regulation of Dorsal. Such studies have identified a set of 25 *cis*-regulatory sequences, which act in concert with Dorsal along the D-V axis (Stathopoulos and Levine, 2004; Markstein *et al*., 2004). Genome-wide studies employing ChIP-chip identified hundreds of binding sequences, including hitherto unknown binding motifs, for Dorsal and associated regulatory proteins in the D-V patterning genes (Sandmann *et al*., 2007; Zeitlinger *et al*., 2007). For instance, apart from Dorsal-binding sequence in the target genes, the motif GCTGGYA was identified in enhancers that control expression in a broad lateral stripe. On the contrary, genes that expressed in the ventro-lateral stripes had CACATGT and RGGNCAG motifs nearby functional Dorsal motifs (Stathopoulos *et al*., 2002; Markstein *et al*., 2002; Markstein *et al*., 2004). The affinity of Dorsal-binding sites within *cis*-regulatory sequences is shown to influence the domain of target-gene expression (Jiang and Levine, 1993). A *twi cis*-regulatory sequence, which normally directs expression to ventral regions of the embryo, exhibited a dorsally expanded expression domain (i.e., the ventro-lateral domain) when the low affinity Dorsal-binding sites were mutated to high affinity ones. Such analysis reinforced the concept of "combinatorial codes" in Dorsal-mediated gene expression.

Main emphasis of this code is that activator and repressor function of Dorsal is modulated by other proteins depending on the high or low affinity of Dorsal for that particular motif (Valentine *et al*., 1998; Jimenez *et al*., 2000). All these studies have pointed to the fact that wide diversity in *κB* motif sequences is not without a reason and perhaps the sequence of the Dorsal-binding motif may have 'a' role in gene regulation and Dorsal function.

Role of κB Motif Sequence and Geometry in Gene Regulation by Dorsal

In recent times, we have started to develop understanding towards the factors that discriminate activator function of Rel proteins from its repressor function. While studying the auto-regulation of Dorsal, we performed motif swap experiment and found that Dorsal interaction with AP-1 was specific for AGAAAAACA motif present in the promoter (Mrinal and Nagaraju, 2010). When this motif was replaced with canonical GGAAATTCC activator motif, co-repressor function of AP-1 was lost. The Dorsal-AP1 complex was not recruited, either *in vitro* or *in vivo*, with the canonical activator motif GGAAATTCC. This indicated that sequence of *κB* motif perhaps had some role to play in Dorsal interaction with its co-regulator (Mrinal and Nagaraju, 2010).

We explored this question further and showed that composition of the *κB* motif sequence has a key role to play in the interaction of co-regulator with Dorsal. We found that all known Dorsal target genes which are repressed by Dorsal, have an A-tract in the binding motif while none of the activator motifs had A-tract. Through molecular simulation studies, we have shown that *κB* core with A-tract (which is present in repressor motifs) is more deformable than activator *κB* core, which always lacks A-tract. We further showed that presence of 'A' or 'T' at the 6^{th} position in the nonameric *κB* motif is critical for imparting activator or repressor function (Mrinal and Nagaraju, 2010; Mrinal *et al*., 2011). While different activator motifs had comparable major groove geometry, the repressor motifs had distinct major groove geometry. Thus, binding of Dorsal to different repressor motifs is decided by the sequence of *κB* motif and the co-repressor (Mrinal *et al*., 2011). This suggests that DNA sequence can induce allosteric changes in the TF to enable its binding to the DNA motif. This led us to conclude that *κB* motifs in *Drosophila* exist in activator and repressor conformations, implying that there is a sequence

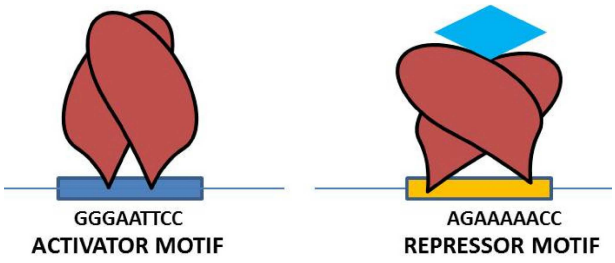

Fig. 2: Rel family transcription factors like Dorsal can take different conformations on different DNA sequences. As a result,on certain motifs Dorsal binds in association with co-regulator while on other sites it binds without cofactors. Thus, recruitment of co-regulator to Dorsal-DNA complex is allosterically controlled

encoded geometry in DNA binding motifs (Fig. 2). Later on, similar findings were made by Wang *et al*. as they found that sequence at the central position of the *κB* motif affects not only transcriptional activity of Rel proteins but also its ability to heterodimerize with other Rel proteins (Wang *et al*., 2012).

Conclusion

Study of Toll pathway in last three decades has greatly enhanced our knowledge of molecular mechanisms in D-V signalling and its regulation. In last few years, research in this field has focussed on understanding the Dorsal gradient formation and its gene regulatory network using high throughput technologies. This has expanded the number of target genes downstream to Toll and brought Dorsal into focus. The precise question to be answered for now is: How does Dorsal decide the qualitative and quantitative nature of target gene expression?

It is evident that ability of Dorsal to induce or repress gene expression is context-based. Understanding this 'context' is very crucial, since this will help in decoding the molecular basis of target gene recognition by transcription factors in general. Differential ability of Dorsal to bind many variants of *κB* motifs leading to different degrees of transcriptional expression of target genes indicates that, there cannot be a simple model of recognition codes of DNA-binding by Dorsal. Flexibility in protein-DNA interactions, selective role of co-regulators and other effects of local and non-local parameters confound straightforward descriptions of DNA base preferences in Dorsal binding (Mrinal *et al*., 2011). Dorsal acts as a global regulator and the co-regulators act in gene specific manner. Since, Dorsal can interact with many co-regulators, this adds tremendously to its DNA-binding diversity (Stathopoulos and Levine, 2004; Markstein *et al*., 2004). Hence, to completely decipher the DNA-recognition codes of Dorsal, high throughput technologies would be required which will help to gain insights into gene targeting *in vivo*. This will also help in understanding the role of DNA induced allostery, co-operative recruitment or combinatorial codes in Dorsal binding which will in turn help us to understand how specificity is imparted in gene regulation.

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