More than meets the dimer: What is the quaternary structure of the glucocorticoid receptor?

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ABSTRACT
It is widely accepted that the glucocorticoid receptor (GR), a ligand-regulated transcription factor that triggers anti-inflammatory responses, binds specific response elements as a homodimer. Here, we will discuss the original primary data that established this model and contrast it with a recent report characterizing the GR–DNA complex as a tetramer.

The glucocorticoid receptor (GR) is a modular transcription factor, organized in three distinct structural and functional domains: the N-terminal domain (NTD), a central DNA-binding (DBD) domain, and a C-terminal Ligand-binding domain (LBD).\(^1\) The GR is expressed in most cell tissues, and is involved in critical biological processes including homeostasis and metabolism. Moreover, pharmacological activation of the GR triggers powerful anti-inflammatory and immunosuppressive actions, making the receptor one of the most targeted proteins for therapy. GR oligomeric manipulation is considered a key aspect in the search of synthetic ligands that would eliminate the side-effects associated with chronic glucocorticoid treatment.\(^2\)

In current dogma, GR is considered to bind to specific glucocorticoid response elements (GREs) as a homodimer. However, primary data references for this model are largely absent in current review articles, or in research articles. This is expected for long-standing, widely accepted models. We recently reported that DNA binding at response elements induces tetramerization of the GR,\(^3\) suggesting that tetramers maybe the final, active chromatin bound form of the receptor. The report\(^3\) presents the first in vivo experiments that directly address the oligomeric state of GR when bound to chromatin. In this brief comment, we will argue that the dimerization model exclusively relies on in vitro data. Furthermore, the recent observations in living cells are, to some extent, consistent with early in vitro data, indicating that new efforts are needed to resolve the long-standing issue regarding the active form of the DNA bound GR.

GR as a dimer: In vitro studies

We refer here to in vitro studies as any experiment that has been performed in a cell-free environment. The first description of GR’s oligomeric status was presented in 1983 by the Yamamoto lab. The authors purified GR from rat liver and analyzed by electron microscopy GR “particles” either bound or unbound to a DNA fragment containing a GRE sequence from the mouse mammary tumor virus (MMTV) promoter region.\(^4\) Based on the size of the particles, these investigators concluded: “…the 94 kD receptor subunits seem to form homotetramers in a DNA-independent manner under these conditions (…) but this has not been confirmed by independent methods.” Later, the Gustafsson group evaluated the relative stoichiometry between GR and DNA.\(^5\) Purified receptor bound to \(^3\)H-triamcinolone acetonide (a GR agonist) is incubated with \(^32\)P-labeled DNA. After glycerol centrifugation, each fraction is analyzed and the relative
radioactive signals are obtained. The authors concluded that only one GR molecule binds a single GRE, suggesting that GR binds to DNA as a monomer. However, the same technique applied on a “stronger” GRE sequence indicated that the activated GR exists as a homodimer when unbound as well as when bound to DNA.\(^6\)

Another commonly used technique to study DNA binding is the electro-mobility shift assay (EMSA). In this case, in vitro translated, GST-purified (usually only the DBD fragment) or endogenous purified receptor is mixed with labeled DNA and the binding products analyzed by polyacrylamide gel. Early efforts identified two distinct shifted bands. These were assigned as monomeric and dimeric complexes, a conclusion perhaps also biased from the dyad symmetry of the GRE sequence.\(^7\) Therefore, results from EMSA experiments suggested that the functional entity that binds to a GRE is a dimer.\(^7,8\) Although some studies reported that the monomer binds first to DNA,\(^7,9,10\) other reports provided evidence for DNA-independent dimer formation.\(^6,8,11–14\) In general, groups that worked with the entire receptor argued for a DNA-independent pathway,\(^8,11,12,14\) whereas those working with the DBD fragment found that it was the monomer that first binds DNA, which in turn favors the binding of the second monomer, a concept known as positive cooperative effect.\(^7,9,10\) The use of other in vitro approaches did not help to solve the controversy. Although DNA-independent positive interactions between in vitro translated GR and cell-extracted immunoprecipitated GR have been observed in solution,\(^15\) other investigators argue that GR exists almost exclusively in a monomeric state.\(^16\) Finally, several early papers suggested that either adjacent GREs\(^17–19\) or unusual GREs\(^20\) could lead to the formation of homo-tetramers. However, when the solution and crystal structures of GR’s DBD were elucidated,\(^21,22\) the community rapidly adopted the idea that the activated GR was in fact a homodimer.\(^23\)

From the structural perspective, only the DBD\(^22\) and LBD\(^24\) domains have been crystalized, although separately. The NTD has eluded crystallization and high-resolution structure, most likely due to its intrinsically disordered domain.\(^25\) The first crystal structure of GR’s DBD bound to DNA revealed a clear dimerization region between the two receptor monomers.\(^22\) Although several studies in the 90s suggested a region outside the DBD could be involved in GR dimerization,\(^10,11,26,27\) the predominant view portrayed the DBD as the exclusive domain responsible for dimer formation. This dogma developed largely because a point mutation in that region (known as the GRdim mutant) was allegedly sufficient to generate a monomeric GR,\(^28,29\) although no direct evidence was provided at the time.\(^30,31\) When the crystal structure of the LBD was reported and a second dimerization region discovered,\(^24\) concerns about the physiological relevance and functional contribution of both domains arose.\(^32\) Some investigators still argue that the LBD dimers are an artifact of crystallization, and that the LBD dimerization of GR is “unlikely.”\(^33\)

Taken together, the evidence from in vitro data appears to indicate that GR is a dimer, although no clear consensus exists as to whether GR dimerizes before or after DNA binding, nor which domains are involved. The clearest results come, however, from studies performed on the DBD fragment alone and not the entire GR protein.

**The in vivo perspective on GR dimerization**

We refer here to in vivo studies as any experiment that has been performed inside living cells, or experiments wherein the biological parameter measured occurred in intact cells but was revealed with an in vitro technique. Inside the cell, in the absence of ligand, the receptor is mostly retained in the cytoplasmic compartment, as a monomer,\(^34\) by being part of a heterocomplex with Hsp90, Hsp70, p23, and immunophils, among others.\(^35\) However, GR overexpression has been reported to induce ligand-independent cytoplasmic dimerization.\(^36\) Since the heterocomplex is necessary for proper folding that allows GR to bind hormone,\(^37\) it is not clear how the GR dimers can still remain associated with the heterocomplex, as they are able to bind ligand and translocate into the nucleus.\(^36\)

Once GR is activated by ligand, it translocates almost completely to the nuclear compartment. Using a nuclear-import deficient receptor mutant in the context of its wild-type counterpart, the Hache lab demonstrated that GR can interact with itself before and/or during retrograde transport.\(^15\) This experiment constitutes the first demonstration that the GR can actually interact with itself in vivo (i.e., inside living cells), many years after the community had already adopted the dimerization paradigm. The Hache group noted at the time: “At present we cannot exclude the potential formation of higher-order
In vitro versus in vivo data: Any room for reconciliation?

In a recent report, we concluded that GR is mostly a tetramer when bound to DNA. This conclusion is based on four independent findings: (i) N&B measurements at an array of response elements show the presence of tetramers; (ii) A mutation that mimics the DNA-bound conformation of GR (P493R) triggers tetramerization in the whole nucleoplasm; (iii) Homo-FRET studies show higher oligomerization states in both nucleoplasm and the array; and (iv) Single-molecule photobleaching experiments detect the presence of greater-than-two-subunit oligomers randomly in the nucleus. Using several mutations, we also reported that tetramer formation depends on the presence of the LBD, is independent of dimerization surfaces, and requires DNA-induced conformational changes in the DBD.

How can these results be reconciled with the previous literature? One of the strongest evidence for GR dimerization is the crystallographic X-ray structure. However, lack of tetramerization of the DBD fragment is not surprising since tetramer formation depends on the LBD. Moreover, the monomer-to-dimer transition observed in EMSA assays is completely consistent with the N&B data once the LBD domain is removed. The NTD–DBD fragment behaves as the DBD behaves in vivo: fully monomeric in the nucleoplasm and fully dimeric at GREs. It is worth mentioning one study, by the Miguel Beato’s group, where they mixed full-length and DBD fragments to form whole-GR/DBD-only heterodimers, strongly suggesting the presence of dimeric forms of the receptor. The in vitro evidence, on the contrary, only points to tetramerization as there are no other direct measurements reported.

If GR binds to DNA as a dimer in vitro and as a tetramer in vivo, then something must be missing in the in vitro system. They are several key elements present in vivo that are absent in the controlled in vitro environment: post-translational modifications, cofactor interactions, nucleosomes, the interphase chromatin landscape, to name a few. Some of these elements could be proven essential for this “next regulatory step” GR seems to have within live cells.

Toward a new model for GR oligomerization

After review of more than 30 years of research, we suggest that the evidence for GR dimerization is not as solid as originally thought. Based on the new N&B...
findings, we propose a new model that attempts to resolve all available data (Fig. 1).

In the absence of ligand, the inactivated GR appears to be fully monomeric inside the nuclear compartment, while their behavior in the cytoplasm could depend on its concentration: At endogenous levels, the GR is most likely a monomer but overexpression of the receptor could lead to ligand-independent dimerization.

When the GR is activated by dexamethasone or its natural ligand corticosterone, dimer formation is initiated before or during the nuclear translocation process. The reported inability of the non-steroid GR ligand Compound A to efficiently promote GR nuclear translocation may be explained by its incapacity to induce GR dimer complexes. Once in the nucleus, virtually all agonist-bound GR molecules are...
in the dimeric form through LBD–LBD and DBD–DBD interactions, although mutational analyses indicate that these dimeric surfaces are not functionally equivalent. In fact, dimerization through the DBD is dependent upon the presence of the LBD. It has also been documented that after specific DNA binding, the DBD changes conformation, potentially favoring DBD–DBD interactions. Hence, the positive cooperative binding between monomers observed in vitro when only the DBD fragment is used may not be a key factor in vivo, and reflects further stabilization of the pre-formed dimers after engaging chromatin. Since GR molecules are already dimeric before binding to DNA, it is the dimer and not the monomer that is the hormone-activated GR entity (Fig. 1).

The GR is allosterically modulated not only by ligand binding, but also by DNA itself. This suggests that GREs do not merely serve as GR docking points, but may also modulate GR activity by altering its conformation. In fact, thermodynamic studies using GR's DBD have shown an induce-fit binding mode to DNA, therefore suggesting at least a two-step, and possibly a multi-step mechanism. We propose that this new conformation triggers a structural re-arrangement in the LBD, promoting the formation of higher order oligomers, predominantly tetramers, through LBD surfaces that are yet to be identified. This phenomenon may be more common than previously thought, as STAT3 has been recently described as transitioning from dimer to tetramers in a DNA-dependent manner. A deeper understanding on the intricacies of GR quaternary structure may help find new strategies in the search for safer glucocorticoids, or at least finally close some roads taken in the past that have led us nowhere. Finally, as combinatorial long-range interactions between regulatory elements play an important role in gene regulation, we speculate the tetrameric nature of some transcription factors such as GR or STAT3 can serve as a platform to bridge different points in the genome.

**Abbreviations**

- ChIP: chromatin immunoprecipitation
- DBD: DNA-binding domain
- EMSA: electro-mobility shift assay
- GC: glucocorticoids
- GR: glucocorticoid receptor
- GRE: glucocorticoid response element
- LBD: ligand-binding domain
- MMTV: mouse mammary tumor virus
- N&B: number and brightness
- NTD: N-terminal domain

**Disclosure of potential conflicts of interest**

No potential conflicts of interest were disclosed.

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