Compare and contrast the small G proteins with heterotrimeric G proteins. Briefly speculate why these different mechanisms might have evolved.

Intro

- Small G proteins – monomeric
- Heterotrimeric – Galpha (homologous to small G), Gbeta, G-gamma
- Both types control a myriad of signalling pathways in cells
- Some mechanisms are similar, some are different

Body

*Give specific examples of small G proteins / heterotrimerics that do each

* Experimental evidence (e.g. NMR?)

1. Structure

	Small G proteins	Heterotrimeric G proteins
Nucleotide binding	Mg2+ (GTP and GDP form)	Same G domain fold as Ras But contains 4 insertions – 1 large helical domain and 3 small insertions Only binds Mg2+ in GTP bound form In GDP bound form, betagamma subunit binds Galpha GDP, thus taking on role of Mg2+
Switch region / mechanism	Thr35 in Switch I and Gly60 in Switch II interact with oxygen atoms on GTP gamma-phosphate; release of gamma-phosphate allows system to relax Others: - Third switch (Ran) - N-terminal switch with interswitch (Arf) Bind to GTP and GDP with equal affinity	3 switch regions – homologous Switch I and II, along with third switch loop that points to nucleotide binding site Arg-Gln hasp connects the three switch regions to lock alpha subunit in Galpha bound conformation → Galpha subunits have preference for GTP binding
Interaction with membrane	Allosteric lobe maintains correct Ras-membrane interaction Prenylation (primary and secondary sites) at C-terminal, CAAX box	Galpha: Gly2 which is myristoylated after N-terminal Met is removed N-terminal Cys is palmitoylated Ggamma: Also contains CAAX box and can be farnesylated or Geranyl-geranylated on cysteine of

		CAAX; AAX residues also cleaved off
PTMs	Prenylation, ubiquitination, phosphorylation	

2. Activation

	Small G proteins	Heterotrimeric G proteins
Activators	GEFs - Open nucleotide binding pocket to reduce affinity of G protein for GDP - "Push-pull mechanism": GEF binds to push switch II, which disrupts contact to phosphates and disturbs the P loop - Switch I is displaced to prevent contacts between Switch I residues and nucleotide - Other effects include occlusion of the ribose binding site All activators appear to disrupt the P loop.	GPCRs - Upon ligand binding, conformational changes in the cytosolic domain of the receptor exposes a hydrophobic cleft by the outward movement of TM5 and 6 - Galpha binds to GPCR via N-terminal alpha helical domain - This induces conformational changes in Galpha, including outward movement of alpha5-beta6 loop, which normally interacts with guanine ring of nucleotide. P loop is also disturbed GBA motif proteins - Monomeric (like GEFs) - Binds in cleft between switch II and alpha3 to disrupt P loop Beta, gamma subunits act cooperatively to promote nucleotide exchange

3. Interaction with effectors

	Small G proteins	Heterotrimeric G proteins
Mechanism of interaction	- Formation of intermolecular beta sheet involving switch I - Disruption of autoinhibition (e.g. Ras disrupts Raf, Cdc42 disrupts WASP) - Dimerisation	- Disruption of autoinhibition (e.g. Galphaq with PLC. transducin with cGMP PDE) - Weaken membrane interaction of Galpha protein (not the case for small G proteins)

	- Nanoclustering	- Direct activation of active site of effector Both Galpha and Gbetagamma can interact with downstream effectors
Effectors	Depends on particular G protein - Ras and Raf - Rho and WASP - Ran and nuclear exporters (e.g. exportins) - Rab and tethering proteins - Arf and COP I vesicles	Depends on particular heterotrimeric - Galphat and cGMP PDE - Galphas and AC - Galphaq and PLC - Betagamma – girk2 ion channels

4. Termination

	Small G proteins	Heterotrimeric G proteins
Intrinsic mechanism	- Gln61 acts as base, positions water molecule in binding site - O of water mediates nucleophilic attack on gamma phosphate - Gln contacts one of gamma phosphate O to stabilise reaction transition state Others:	- Contains its ow Arg finger -> Galpha hydrolyses GTP 100x faster than small G proteins
Terminators	GAPs - Arg finger has positive charge, neutralises buildup of negative charge on beta and gamma phosphate O - Stabilises catalytic Gln61 - Arg positioning stabilised by secondary residue (e.g. Arg, Lys) - Contacts switch regions Others: co-activator GAPs and GEFs can be recruited by the same receptor (e.g. PDGFR recruits SOS and p120, the GEF and GAP for Ras, respectively)	RGSs - Contacts switch regions – holds them in correct orientation so Arg finger can act in nt binding site - Can stabilise catalytic moieties e.g. Gln, water Some effector proteins - PLC Asn260 interacts with Gln209 of Galphaq to stabilise it; Asn260 interacts with Glu212 of Switch I - cGMP PDE orientates Switch I and II to be in correct orientation for interaction with RGS9; also stabilises R2014 to H bond to G199, thus stabilising catalytic Gln200

5. Interaction with membrane

	Small G proteins	Heterotrimeric G proteins
Structural elements	- Via prenyl groups - Nanoclustering - Allosteric lobe	- Via prenyl groups - Via betagamma (gamma has prenyl group) - Do not appear to exhibit nanoclustering or dimerisation in the membrane (apart from with GPCRs)

6. Reasons for evolution of different mechanisms

Speculation

- Due to structural divergence between the small G and heterotrimeric G proteins, activation mechanism also had to be differentiated
- Reduce cross-talk between the two pathways so that there is some independence at the activation step (though cross-talk does occur in later parts of the pathway)
- Different functions

Conclusion

- Both small G proteins and heterotrimerics exhibit some similarity, but there are many differences
- Both G proteins show great diversity in the mechanisms of signalling and functions
- Integrate signals
- Highly important in the cell
    - Targets of drug discovery campaigns e.g. Ras
- Exemplars of structure-function relationships